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Influences of Anadromous Spawning Behavior and Optimal Environmental Conditions Upon Striped Bass (*Morone saxatilis*) Year-Class Success[1]

R. E. ULANOWICZ

University of Maryland, Chesapeake Biological Laboratory, Solomons, MD 20688, USA

AND T. T. POLGAR[2]

Martin Marietta Corporation, Environmental Center, Baltimore, MD 21227, USA

ULANOWICZ, R. E., AND T. T. POLGAR. 1980. Influences of anadromous spawning behavior and optimal environmental conditions upon striped bass (*Morone saxatilis*) year-class success. Can. J. Fish. Aquat. Sci. 37: 143–154.

An analysis of the spatial and temporal abundance patterns of developing striped bass (*Morone saxatilis*) ichthyoplankton stages in the Potomac Estuary, including a Markovian description of transport, indicates that annual differences in the distribution of spawning fish are not likely to account wholly for the wide fluctuations in year-class success. Year-class success can be decomposed into the product of two factors — one extrinsic, acting upon the eggs and larvae, and the other, a behavioral property of the adult stock. The effect of extrinsic environmental conditions upon year-class success can be measured relative to the maximum computed survival of eggs to post-finfold larvae among all locations and times during a given year. Apparently, the behavior of the spawning adult fish is not well matched with the environmental conditions favorable to ichthyoplankton survival, and therefore, the actual spawning distribution yields only a fraction (the spawning fitness) of the maximum production possible during that year. Spawning fitnesses were estimated to be small (<0.02 out of 1.0) and varied by less than a factor of two over the three seasons observed. In contrast, the year-class success as measured by post-finfold production differed 35-fold over the same 3 yr. It appears most likely that the large range in success is due primarily to the extrinsic, density-independent environmental factors which determine the optimum survivals in combination with spawning behavior. However, no strong case can be made for behavioral compensation by spawning fish to offset changes in the annual optimum survival conditions for ichthyoplankton.

Key words: anadromous, ichthyoplankton development, Markovian transition probabilities, optimal survival, spawning behavior, spawning fitness, striped bass, transport model, year-class success

[1]Contribution No. 876 of the University of Maryland's Center for Environmental and Estuarine Studies.

[2]Also affiliated with the Chesapeake Biological Laboratory, University of Maryland, Solomons, MD 20688, USA.

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Une analyse des modalités de l'abondance spatiale et temporelle de stades ichtyoplanctoniques de bars d'Amérique en voie de développement dans l'estuaire du Potomac, y compris une description markovienne du transport, indique que les différences annuelles de distribution des reproducteurs ne peuvent probablement pas expliquer complètement les grandes fluctuations d'abondance d'une classe annuelle à l'autre. Le succès d'une classe annuelle peut être décomposé comme résultant de deux facteurs — un facteur extrinsèque agissant sur les oeufs et les larves, et l'autre, une propriété de comportement du stock adulte. L'effet de conditions ambiantes extrinsèques sur le succès d'une classe annuelle peut être mesuré en relation avec la survie maximale calculée depuis l'oeuf jusqu'à la larve avec repli cutané des nageoires, à tout endroit et temps d'une année donnée. Le comportement des reproducteurs, semble-t-il, ne s'accorde pas très bien avec les conditions ambiantes favorables à la survie de l'ichtyoplancton. Comme conséquence, la distribution effective de la fraie ne produit qu'une fraction (aptitude reproductrice) de la production maximale possible cette année-là. On estime que l'aptitude reproductrice est faible (<0,02 sur 1,0) et varia de moins de un ou deux facteurs durant les trois saisons d'observation. Par contraste, le succès d'une classe annuelle mesurée par production de larves au stade postrepli varia de 35 fois durant les mêmes 3 années. Il semble très probable que la gamme étendue de succès est surtout due à des facteurs ambiants extrinsèques, indépendants de la densité, qui déterminent la survie optimale, auxquels s'ajoute le comportement reproducteur. On ne peut toutefois pas prouver que les reproducteurs ont un comportement compensatoire pour contrebalancer les changements de conditions optimales annuelles de survie de l'ichtyoplancton.

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THE most significant mortalities for many fish populations occur during the early planktonic or juvenile life stages. In most fish populations, year-class strength is determined by the end of larval development, and all age-groups remain roughly proportional to the annual abundance of the fingerling cohort (Chadwick et al. 1977). If the developmental process is biologically controlled through density-dependent mechanisms such as competition, predation, or cannibalism, adult population levels reach long-term equilibrium values and age structure stabilizes. However, when annual survival during early development is significantly affected by extrinsic, density-independent environmental conditions, adult population levels display large annual fluctuations, and the age structure becomes highly time-dependent. Striped bass populations seem to display the latter behavior, as indicated by dominant year-classes controlling catches in the fishery (Koo 1970). Catch records show population levels to be unstable, lacking a definable, long-term equilibrium point (Van Winkle et al. 1979). Knowledge of environmental conditions in the spawning–nursery area during years with very large or very low survivals is, therefore, of utmost importance to anyone seeking to understand and manage such a fishery. This is especially true in instances when the management objective is the protection of early life stage habitats instead of the traditional maximization of yield.

For species with spawning and nursery areas very small in geographical extent, the problem of larval habitat protection can be resolved into monitoring conditions at the spawning and nursery site over a series of seasonal spawning events, and then quantitatively correlating these spawning stock conditions and environmental factors with the subsequent production figures of young adults. A more likely situation, however, is that temporally prolonged spawning and early development occur over a diffuse area having significant environmental gradients. Further complications arise when one realizes that a given individual spawned at a certain site in the area may be transported through differentially favorable environments within the region before either succumbing or being recruited into the population as a young adult. An investigator with data on spatial and temporal abundances of various egg, larval, and juvenile stages is, thus, faced with the task of deconvoluting the effect of passive transport upon the observed abundance patterns, in order to estimate the most likely times and places where greater or lesser mortalities (or, inversely, lesser or greater survivals) have occurred.

Having assessed the successive survival patterns through various stages, the nontrivial next step is to answer the question, "What are the key sites and times within the general nursery area for which survival of the various early stages contributes most to recruitment into the population?" If the ultimate goal of the manager is the maintenance of the reproductive capability of the species through the conservation of spawning–nursery habitats, it is evident that the crucial times and places must be protected from excessive perturbation by man.

A closely related, but separate question, a manager might pose is, "At what time, place, and life stage do larvae and juveniles have the greatest probability of contributing to the adult stock?" The answer to this

query could be the foundation of an attempt to artificially increase the production of the fishery. Such considerations can dictate how long to raise larvae and juveniles under artificial conditions before releasing them at some optimal time and location to supplement natural reproduction. Of more theoretical interest is the corollary question, "How efficient was a given spawning season; or, what is the spawning fitness of the population?" By comparing the actual spatial and temporal distribution of production with the hypothetical production of the same number of eggs released at optimal locations and times, one can obtain a quantitative measure of the fitness of the observed spawning distribution. The changes in fitness from year-to-year might shed some light on the adaptive significance of specific spawning distribution and behavior in the adult population.

The techniques for approaching answers to these questions form the subject matter of this article. The methods presented here were developed to analyze the results of an extensive striped bass (*Morone saxatilis*) population study in the Potomac estuary during the spawning seasons 1974–76. Ichthyoplankton, juvenile, and spawning stock surveys comprised the biological elements in this study. Full details on the study area, sampling procedures and abundance estimates can be found elsewhere (Polgar et al. 1976; Polgar 1977; Boynton et al. 1977; Mihursky et al. 1976; Setzler et al. 1978) and will be described only briefly below. Full explanations of the uniform age-class model and of the spatial transition probabilities are in preparation (T. T. Polgar and P. A. Souza unpublished data). Only the points necessary for comprehension of the spawning fitness analysis are repeated here.

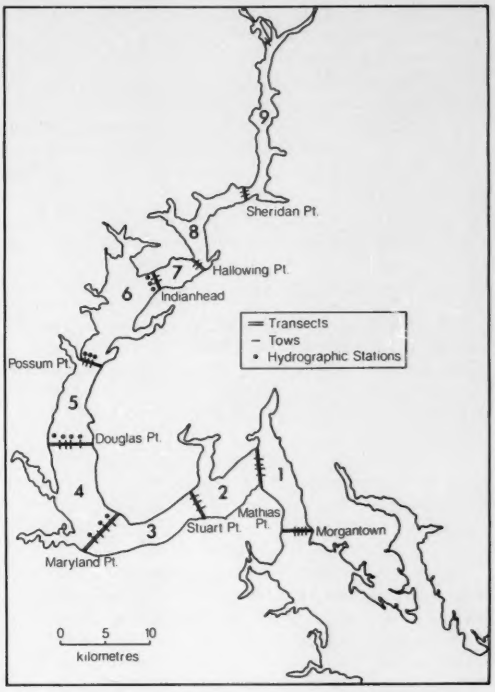

FIG. 1. Study area, showing estuarine segment designations used in the analysis.

Study Area and Sampling Procedures

The spawning–nursery ground under study is the upper Potomac Estuary, from 69 km above the mouth, in the oligohaline region, to 151 km, well into the freshwater region and above the major area of spawning activity. The sampling design description is specific to the 1974 survey and no significant alterations in design were made in subsequent years. Twelve transects were established along the study area and spaced so as to divide the estuarine reach into equal volume (2×10^8 m^3) segments, each having a length greater than a single tidal excursion (see Fig. 1). A single boat sampled all transects weekly throughout the 17-wk duration of the spawning and developmental periods. Several tows were taken perpendicular to each cross-stream transect at 38 separate locations (total for all 12 transects) as shown on Fig. 1.

All ichthyoplankton were identified to species (where possible) and were counted for each tow. Striped bass ichthyoplankton were further classified into four life stages: eggs, yolk-sac larvae, finfold larvae, and post-finfold larvae. Similar ichthyoplankton surveys were carried out during the 1975 and 1976 spawning seasons.

During the beginning of the 1974 spawning season, 13 current meter stations were established along the Maryland Point, Douglas Point, Possum Point, and Indian Head transects (Fig. 1). Divided among the 13 stations were 21 Braincon Histogram recording current meters. Current speed and direction were automatically recorded at 10-min intervals over a 15-d period from April 23 through May 8. These hydrodynamic data were intended for use in a vertically averaged, two-dimensional model of ichthyoplankton dispersion, but also were employed in estimating the one-dimensional longitudinal dispersion and advection characteristics necessary to calculate the spatial transition probabilities required in this analysis.

Abundance and Survival Patterns

Estimation of Abundances

Data from the field collections and the laboratory sorting provided information on the catches in each tow. Each tow was associated with a transect and a week of the season, and was characterized by the volume of water strained in cubic metres and the numbers of each life stage of striped bass caught. It was necessary to transform these numbers into estimates of abundance of each life stage within each of the estuarine segments.

Pyne (1979) demonstrated that the minimum variance, unbiased estimator of the average density for a series of tows from the same distribution of organisms

collected in varying tow volumes was simply the sum of all the numbers of a given age-class from all tows divided by the sum of the respective volumes sampled. A given segment was bounded by two transects and the density estimator for the segment was obtained by extending the sums of number and volume over all tows made on the two boundary transects. The abundance of life stage k during week j in segment i was calculated by multiplying the corresponding average density by the river volume of segment i (Polgar 1977). The derived abundance is designated by the symbol $A^k{}_{ji}$.

Spatial Transition Probabilities

It was clear from the outset that because the spawning and nursery area is distributed in space, information was necessary on how the ichthyoplankton are transported throughout the study area. Transport, even in the fresher portions of the estuary, like most large-scale fluid flows, is known to contain a stochastic element, termed dispersion, which is the net result of small scale random motions viewed on a larger scale. Advection, or net displacement, is also part of the transport process, but is deterministic, given river geometry and freshwater inflow. Hence, it is impossible to say with certainty where an organism originating in segment i will be found at the end of a week. Rather, it is necessary to speak of the probability, P_{il}, that a given animal in segment i during one time interval will appear in cell l during the next time interval, assuming that the organism does not suffer mortality nor develop into the next stage during the interim. That is, P_{il} is the transition probability applicable to an organism transported from location i to location l due to weekly spatial movement in the absence of mortality.

In what follows, P_{il} will refer to transitions between segments of a linear chain during fixed time intervals, but the reader should bear in mind that this in no way restricts the application of the subsequent methodology to one-dimensional situations. Stationary but spatially dependent transport within any two- or three-dimensional spatial network can be characterized by a Markovian matrix of P_{il}'s so long as the cells are denumerable.

Eggs and larvae of the age-classes under consideration are assumed to have no appreciable ability to overcome the water movements. They are assumed to ride passively with the currents, being considered planktonic in these life stages. The assumption of passive transport during the first 8 wk is consistent with approximations made in some other striped bass models (Wallace 1975). Graham et al. (1972) suggest how herring larvae might maintain their position by vertical migration to take advantage of two-layer estuarine net flows, but during the striped bass spawning seasons the region described herein is almost completely upstream of the estuarine segment in which two-layer flow is normally observed. Hence, an appropriate hydrodynamic model for the dispersion of a chemical species may be employed to calculate the transition probabilities. Polgar et al. (1975) described how Hunter's (1975) one-dimensional mass, momentum, and species balances are calibrated against the current meter data recorded during this study.

Briefly, Hunter's model is one-dimensional and nonlinear, including both the dynamic and kinematic equations taken from Harleman and Lee (1969), Harleman (1971), and Thatcher and Harleman (1972). The geometric parameters describing the river were taken from statistics compiled by Cronin and Pritchard (1975) and Cronin (1971). The model was driven at the landward end by freshwater and tidal flows. Freshwater flow was taken to be the average input (over the study period) as measured at the Chain Bridge gauging station in Washington, D.C. The amplitudes of the tidal flows were taken to be transect averages of the values of curve-fitted current meter records at Indian Head. The seaward boundary was driven by predicted tidal heights initially, and thereafter the phases and amplitudes were tuned until the net velocities, tidal velocity amplitudes, and tidally averaged exchange coefficients reasonably approximated estimated values from measurements in 1974.

Transition probabilities were estimated from the calibrated model by placing a known initial concentration only in segment i and running the model over 14 tidal cycles (approximately 1 wk). The ratios of the final concentrations in the segments to the initial amount in segment i determined the elements of P_{il}. This procedure was repeated with each segment as the origin of organisms. Transition probabilities for 200 and 500 m^3/s riverine inputs are shown in Table 1. The rows do not sum to unity in all cases because of the finite probability of transport out of the region. The lower flow characterized the 1974 and 1976 seasons, whereas 500 m^3/s prevailed in 1975. In the following analysis, the transition probabilities will be assumed stationary over the 17 or so weeks of each of the three study periods. Constancy of transition probabilities is a mathematical convenience permitting the analytical solution which follows. Variations in the flow regime are being investigated.

Survival Estimates

Thus far a model has been described to account for conservative spatial–temporal transitions; it remains for the uniform age-class model to represent how an organism enters the succeeding life stage or dies in the process. It is possible to project an expected abundance pattern forward in time as though mortality did not occur. These projections should be greater than the observed abundances for the following week, and the survival can be estimated from comparison of observed and projected abundances, given some hypotheses about the rates of development.

In reality, transport and development occur continuously. The fact that populations can only be sampled

TABLE 1. Spatial transition probabilities for weekly periods.

	1	2	3	4	5	6	7	8	9
				A) Probabilities at 200 m³/s riverine input					
1	0.195	0.146	0.045	0.010	0.001	0.000	0.000	0.000	0.000
2	0.250	0.200	0.145	0.047	0.010	0.001	0.000	0.000	0.000
3	0.156	0.239	0.196	0.146	0.046	0.009	0.001	0.000	0.000
4	0.088	0.165	0.216	0.254	0.148	0.049	0.009	0.001	0.000
5	0.034	0.080	0.158	0.279	0.261	0.125	0.031	0.005	0.000
6	0.006	0.018	0.062	0.184	0.319	0.262	0.112	0.029	0.002
7	0.000	0.002	0.013	0.065	0.207	0.306	0.264	0.123	0.015
8	0.000	0.001	0.004	0.029	0.130	0.268	0.336	0.199	0.030
9	0.000	0.000	0.002	0.013	0.078	0.210	0.362	0.271	0.057
				B) Probabilities at 500 m³/s riverine input					
1	0.125	0.075	0.015	0.001	0.000	0.000	0.000	0.000	0.000
2	0.200	0.130	0.070	0.012	0.001	0.000	0.000	0.000	0.000
3	0.153	0.204	0.120	0.061	0.011	0.001	0.000	0.000	0.000
4	0.143	0.228	0.205	0.159	0.051	0.009	0.001	0.000	0.000
5	0.087	0.168	0.223	0.256	0.135	0.036	0.004	0.000	0.000
6	0.027	0.070	0.155	0.293	0.283	0.124	0.020	0.002	0.000
7	0.005	0.019	0.068	0.204	0.344	0.259	0.080	0.012	0.000
8	0.002	0.008	0.036	0.139	0.322	0.326	0.138	0.025	0.000
9	0.001	0.004	0.022	0.100	0.289	0.357	0.186	0.037	0.000

at discrete time intervals, however, makes information on the detail of this continuous change inaccessible. Therefore, the following three assumptions will introduce errors which we feel are commensurate with, but not greater than, the error due to the discrete sampling rate:

1) The members within an age-class are uniform in age between any given pair of sampling intervals.
2) Death can occur only at the time of development into the next age-class and after transport has been calculated.
3) Developmental rate is uniform and proportional to the number of generations occurring between sampling periods.

Optimally, of course, one wishes to minimize the sampling interval with respect to life stage durations, but economic constraints forced the choice of the weekly sampling rates.

If no maturation or death occurred from one sampling time to the next, the observed abundance of age-class $k+1$ in segment l at the next sampling time would be equal to the product:

$$(1) \quad A^{k+1}{}_{j+1,l} = \sum_{i=1}^{nseg} A^{k+1}{}_{jl} P_{il}$$

where *nseg* is the number of river segments.

If organisms are allowed to mature from one age-class to the next, the estimate in equation (1) would change in two ways. First, there would be a gain of organisms from age-class k entering into $k+1$. If t^k represents the average time an individual spends in class k, then after the sampling interval, t^*, the fraction t^*/t^k of those organisms with age k arriving at segment l would develop into class $k+1$ organisms, i.e. the gain would be:

$$\frac{t^*}{t^k} \sum_{i=1}^{nseg} A^k{}_{jl} P_{il}$$

Similarly, the amount lost to age-class $k+2$ due to development would be:

$$\frac{t^*}{t^{k+1}} \sum_{i=1}^{nseg} A^{k+1}{}_{jl} P_{il}$$

Making these two corrections, the expected abundances without mortality become:

$$(2a) \quad A^{k+1}{}_{j+1,l} = \sum_{i=1}^{nseg} A^{k+1}{}_{jl} P_{il} + \frac{t^*}{t^k} \sum_{i=1}^{nseg} A^k{}_{jl} P_{il} - \frac{t^*}{t^{k+1}} \sum_{i=1}^{nseg} A^{k+1}{}_{jl} P_{il}$$

or,

$$(2b) \quad A^{k+1}{}_{j+1,l} = \frac{t^*}{t^k} \sum_{i=1}^{nseg} A^k{}_{jl} P_{il} + \frac{t^{k+1} - t^*}{t^{k+1}} \sum_{i=1}^{nseg} A^{k+1}{}_{jl} P_{il}$$

Mortality is inevitable, and only the fraction $S^k{}_{j+1,l}$ of those organisms developing into class $k+1$ survive the transition from class k. Whence,

$$(3) \quad A^{k+1}{}_{j+1,l} = S^k{}_{j+1,l} \frac{t^*}{t^k} \sum_{i=1}^{nseg} A^k{}_{jl} P_{il} + \frac{t^{k+1} - t^*}{t^{k+1}} \sum_{i=1}^{nseg} A^{k+1}{}_{jl} P_{il}$$

become the elements of the time–space matrix of forecasted abundances of stage $k+1$ organisms during interval $j+1$. This forecast includes both the develop-

mental and mortality processes. Equation (3) is the fundamental iterative relation upon which the remainder of this paper will be built.

If the abundances are available from field data, and the transition probabilities are known from Table 1, equation (3) may be solved to give an estimate of the stage survivals:

$$(4)\quad S^k{}_{j+1,l} = \left(A^{k+1}{}_{j+1,l} - \frac{t^{k+1} - t^*}{t^{k+1}} A^{k+1}{}_{jl} P_{il}\right) \Bigg/ \left(\frac{t^*}{t^k} \sum_{i=1}^{nseg} A^k{}_{jl} P_{il}\right)$$

A full description and analysis of the abundance and survival patterns for striped bass in the Potomac Estuary is in preparation (T. T. Polgar and P. A. Souza unpublished data).

Spawning and Nursery Production Analysis

In managing the nursery area, it is quite useful to know the abundance and survival patterns. In the absence of further analysis, one may guess that regions and times with high abundances and survivals are critical to the yield of the fishery and should be protected. But such guesses are apt to be imprecise. For example, it is altogether possible that a large abundance of eggs with a high survival into the yolk-sac stage is observed, and the conclusion is drawn that that particular time in the breeding season and reach of the river should be given top priority for protection. It may turn out, however, that these yolk-sac larvae suffer heavy mortality in the next transition and actually make less of a contribution to the next life stage than a more moderate spawn at a different place and time. The point here is that the manager is not in the business of protecting the young per se, but of maximizing the contribution of the spawning–nursery area to the adult stock.

In the particular survey under discussion, no systematic data exist on juvenile stages between the post-finfold and the adult fish, hence an analysis of the ultimate contribution of the observed abundances to the fisheries stocks cannot easily be made. The post-finfold juveniles, however, have attained some mobility, and it is probably reasonable to assume that subsequent mortality rates, while not negligible, do not approach the magnitude of that which has already transpired. For our purposes, it is reasonable to assume that year-class strength is defined by post-finfold stage abundances, and that compensatory mechanisms affect only earlier stages. As a consequence, the relative size of any particular year-class can likely be judged by its total production of post-finfold larvae.

Under the assumptions culminating in equation (3), it is possible to write an expression for the total production of post-finfold juveniles.

Numbering the eggs, yolk-sac, finfold, and post-finfold age-classes one through four, respectively, the total post-finfold production, T, becomes:

$$(5)\quad T = \frac{t^*}{t^3} \sum_{l,i=1}^{nseg} \sum_{j=2}^{nwks} S^3{}_{jl} P_{il} A^2{}_{j-1,i}$$

where *nwks* refers to the number of weekly samples in the time series. Equation (4) describes the total production of post-finfold fish in terms of the abundances of the finfold individuals. It is also desired to express T in terms of egg and yolk-sac abundances. With some patience it is possible to substitute successively the iterative equation (3) into the finfold abundance terms in equation (5) and express the total production in terms of the yolk-sac abundances:

$$(6)\quad T = \frac{t^*}{t^3}\frac{t^*}{t^2} \sum_{p,l,q=1}^{nseg} \sum_{v=1}^{nwks-2} \sum_{j=v+2}^{nwks} \times \left\{\left(\frac{t^2 - t^*}{t^2}\right)^{v-1} S^3{}_{jl} S^2{}_{j-v,p} (P_{pl})^v P_{qp} A^2{}_{j-v-1,q}\right\}.$$

Repeating this substitution process, one derives an expression in terms of egg abundances:

$$(7)\quad T = \frac{t^*}{t^3}\frac{t^*}{t^2}\frac{t^*}{t^1} \sum_{p,m,l,q=1}^{nseg} \sum_{v=1}^{nwks-3} \sum_{w=1}^{v} \sum_{j=v+3}^{nwks} \times \left\{\left(\frac{t^2 - t^*}{t^2}\right)^{w-1} \left(\frac{t^1 - t^*}{t^1}\right)^{v-w} \times S^3{}_{jl} S^2{}_{j-w,m} S^1{}_{j-v-1,p} (P_{ml})^w (P_{pm})^{v-w+1} P_{qp} A^1{}_{j-v-2,q}\right\}.$$

The subscripts m, p, q, v, and w are dummy indices defined solely to prevent ambiguities from arising during multiple summations. Superscripts after a right parenthesis indicate an algebraic power.

The derivation of equations (5), (6), and (7) has not been an idle algebraic exercise, since the variation of T with any particular survival event or abundance is contained explicitly in these equations. In particular, it is useful to ask what the variation in a given survival event, say $S^k{}_{tr}$, might mean. One expects the survivals will depend primarily on extrinsic quantities such as water quality or food availability. Anthropogenic point-source perturbations, such as power plant cooling system effects or sewage discharges are likely to impact the survivals. It is desirable, therefore, to calculate the sensitivity coefficients of T with respect to a survival event through each of the developmental stages into the post-finfold stage as

$$(8)\quad \frac{\partial T}{\partial S^3{}_{tr}} = \frac{t^*}{t^3} \sum_{i=1}^{nseg} P_{ir} A^3{}_{t-1,i}$$

$$(9)\quad \frac{\partial T}{\partial S^2{}_{tr}} = \frac{t^*}{t^3}\frac{t^*}{t^2} \sum_{l,q=1}^{nseg} \sum_{v=1}^{nwks-t} \left\{\left(\frac{t^2 - t^*}{t^2}\right)^{v-1} \times S^3{}_{t+v,l} (P_{rl})^v P_{qr} A^2{}_{t-1,q}\right\},$$

$$(10)\quad \frac{\partial T}{\partial S^1_{tr}} = \frac{t^*}{t^3}\frac{t^*}{t^2}\frac{t^*}{t^1}\sum_{m,l,q=1}^{nseg}\sum_{v=1}^{nwks-t-1}\sum_{w=1}^{v} \times\left\{\left(\frac{t^2-t^*}{t^2}\right)^{w-1}\left(\frac{t^1-t^*}{t^1}\right)^{v-w} \times S^3_{t+v+1,l}S^2_{t+v+1-w,m}(P_{ml})^w(P_{rm})^{v-w+1}P_{qr}A^1_{t-1,q}\right\}.$$

Comparison of equations (8), (9), and (10) with (5), (6), and (7), respectively, reveals that the sensitivity of coefficients may be interpreted as the sequential contributions to the total production of post-finfold animals that would come from all organisms participating in each corresponding stage-to-stage survival event S^k_{tr} if they all had survived that particular transition. Since only the fraction S^k_{tr} actually survive from each previous stage to the post-finfold stage, the contribution of time t and location r to the post-finfold production,

$$(11)\quad V^k_{tr} = S^k_{tr}\frac{\partial T}{\partial S^k_{tr}}$$

is a proper measure of the significance the conditions that time t and river segment r have upon the total production. Conversely, it is also a measure of the vulnerability of the production to any disturbance at the given location and time. A useful check upon one's calculations is the relation

$$(12)\quad T = \sum_{t=1}^{nwks}\sum_{r=1}^{nseg} V^k_{tr} \quad \text{for } k = 1, 2, 3.$$

Relation (12) highlights the fact that the V^k_{tr} also trace the histories of organisms which eventually survived as post-finfold larvae. One may, for example, use V^1_{tr} to map out the pattern of eggs which developed into post-finfold larvae.

Survival Probabilities Computed Through to the Early Juvenile Stage

The previous section dealt with changes in extrinsic factors affecting survival. It is possible to repeat the analysis to examine the effects of directly interferring with the abundances at various locations and times during any stage of development. Such a situation would be encountered, for instance, if larvae or juveniles from a hatchery operation were being introduced into a natural nursery area. The optimal release strategy will depend upon balancing the cost of raising animals to a given age, then transporting them to a given location at a specified time, against the highest probability that an organism of stage k released at time t in location r will survive to contribute to the adult stock.

To address the latter issue in this optimization, one must compute how T varies with a change in abundance, i.e. $\partial T/\partial A^k_{tr}$ must be calculated from equations (5), (6), and (7) to get:

$$(13)\quad \frac{\partial T}{\partial A^3_{tr}} = \frac{t^*}{t^3}\sum_{l=1}^{nseg} S^3_{t+1,l}P_{rl}$$

$$(14)\quad \frac{\partial T}{\partial A^2_{tr}} = \frac{t^*}{t^3}\frac{t^*}{t^2}\sum_{p,l=1}^{nseg}\sum_{v=1}^{nwks-t-1}\left\{\left(\frac{t^2-t^*}{t^2}\right)^{v-1} \times S^3_{t+v+1,l}S^2_{t+1,p}(P_{pl})^vP_{rp}\right\},\ \text{and}$$

$$(15)\quad \frac{\partial T}{\partial A^1_{tr}} = \frac{t^*}{t^3}\frac{t^*}{t^2}\frac{t^*}{t^1}\sum_{m,l,p=1}^{nseg}\sum_{v=1}^{nwks-t-2}\sum_{w=1}^{v} \times\left\{\left(\frac{t^2-t^*}{t^2}\right)^{w-1}\left(\frac{t^1-t^*}{t^1}\right)^{v-w} \times S^3_{t+v+2,l}S^2_{t+v+2-w,l}S^1_{t+1,p}(P_{ml})^w(P_{pm})^{v-w+1}P_{rp}\right\}.$$

In words, the derivatives described in equations (13)–(15) compute the addition to the total post-finfold or early juvenile production, T, which arises from a unit increase in abundance of organisms of stage k at time t and location r. Any of these derivatives may be recognized as the probability of a single organism in any stage surviving into the post-finfold stage. One may use equations (13)–(15) to map out times and locations of varying survival probabilities for each life stage.

The Fitness of Natural Spawning

With the ultimate survival probabilities now at hand it is interesting to focus upon the fate of the eggs originally spawned. While eggs are distributed over space and time, there will usually be particular spawning times and locations from which the eggs would have the greatest chances for ultimate survival. If the spawning stock were well adapted to conditions controlling the survival of the young, they would tend to have spawned all their eggs under optimal circumstances, and the total production under such conditions could be estimated by multiplying the total number of eggs spawned over the entire season by the optimal survival probability for that spawning season. The ratio of the actual production of post-finfold larvae to this hypothetical maximum production provides a measure of how fit the spawning population was in its behavior to produce viable offspring under the environmental conditions which prevailed.

There is no reason to expect a priori that this spawning fitness measure will be extremely high. Spawning fish would have evolved, at most, only weak responses to specific environmental conditions, such as water quality or food plankton productivity that would ensure survival of the early stages. More likely, they are adapted to spawn according to environmental cues which result in juvenile distributions most likely to succeed during the average conditions of the past several hundred or so spawning seasons. A key question might be how the spawning fitness varies with spawning potential, or population fecundity, to discern whether or not a density-dependent compensatory mechanism is at work to stabilize long-term population levels. As we shall see from the subsequent analysis, the answer to this question appears to be negative.

Analysis of Spawning Seasons

The three spawning seasons surveyed differed significantly in terms of overall survival of eggs into postfinfold larvae. Using the computational method explained in Polgar (1977), the overall survival was poorest in 1974 (5.92×10^{-5}), greatest in 1975 (2.07×10^{-3}), and intermediate in 1976 (6.97×10^{-4}).

With survivorships differing by 35-fold, the question immediately arises whether any significant change in the spawning pattern could account for these differences. To address this issue, we performed the calculations specified by equations (4), (8), (9), (10), (13), (14), and (15) upon the egg and larval abundance patterns for the 3 yr. The 200 m^3/s transition matrix was used in the 1974 and 1976 calculations (Table 1A), whereas the 500 m^3/s probabilities were used to represent the 1975 flow regime (Table 1B). Development times t^1, t^2, t^3, t^4 of 2, 12, 11, and 30 d, respectively, were used for the four stages and a sampling interval, t^*, of 7 d was the actual approximate period between surveys. Survivals should vary between zero and unity. When calculated from the raw data, however, a fraction of the survivals exceeded these bounds due to random and systematic sampling errors. The survivals were artificially constrained within their natural bounds by setting negative survival values equal to zero, and those over one are reduced to unity. The error this convention engenders in the absolute values of the results can be assessed by evaluating how the check according to equation (12) is violated. Using such a check on balances, the total productions from each life stage seem to be in error by about 25%, but we do not believe that the qualitative character of survivorship distributions has been excessively warped in space or time. The results presented in the following figures were normalized with respect to their totals to yield percentage distribution patterns.

The significant computational results are displayed in Fig. 2, 3, and 4. On each graph, time is displayed in weeks along the abscissa beginning with the first week in April, while spatial position is arranged on the ordinate according to river segment number. Upstream is toward the top of each graph. Since a strong correlation exists between time, temperature and photoperiod throughout the estuary, some may find it more convenient to regard the abscissa time variable as an aggregate indicator of conditions controlling the spawning distribution.

Figures 2a, b, c display the egg distribution patterns observed during the three seasons. Contrasting the best and the worst seasons, the 1975 spawn is seen to be the most tightly clumped spatially and temporally. In 1974 spawning occurred earlier and was more in evidence further downstream. Nonetheless, the major portion of both spawns was contained in the quadrant defined by weeks 4 and 5 and segments 5 and 6. Spawning during 1976 was the most dispersed of all 3 yr, but even then, the aforementioned quadrant was an active locus of egg release. To summarize, the centroid of spawning appears to be roughly the same in all seasons, whereas the egg pattern dispersion does differ from season to season.

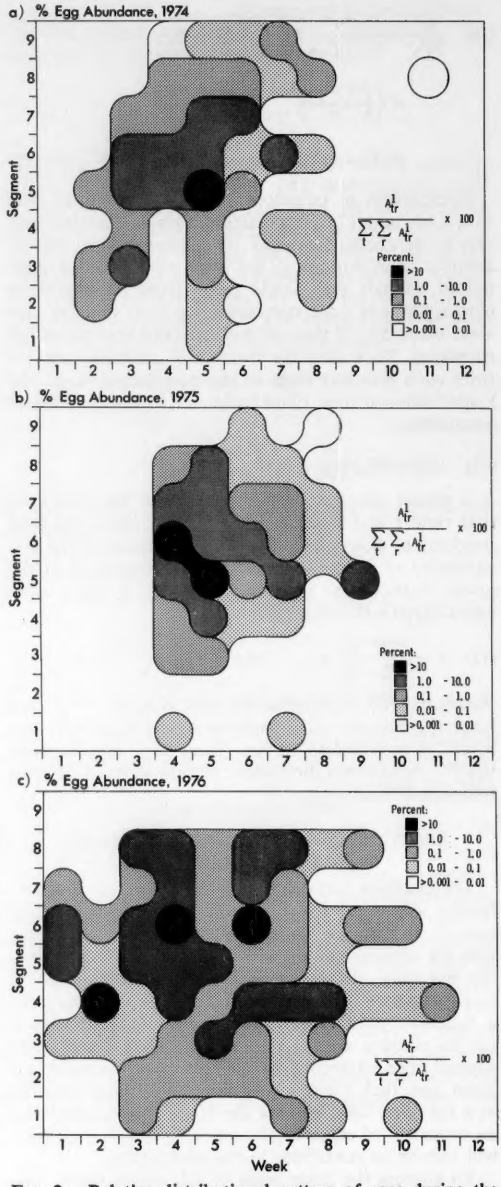

FIG. 2. Relative distributional pattern of eggs during the a) 1974 spawning season, b) 1975 spawning season, and c) 1976 spawning season.

By employing equations (11) and (12) it becomes possible to map out where and when the surviving post-finfold larvae probably originated as eggs, i.e. one may sketch the pattern of "successful" eggs. These percentile distributions are depicted in Fig. 3a, b, and c. Comparing these patterns with their counterparts in Fig. 2 provides only minor contrasts. In 1974 the most successful spawning took place upstream and subsequent to the major egg pulse at Douglas Point. In 1975 there was no apparent spatial shift between the major spawn and the successful eggs, but survivors did appear in greater numbers from the eggs spawned later in the season. The tendency for surviving eggs to come from later spawn is repeated again in 1976. Despite obvious differences in the patterns of success, there remains good overlap in survivorship among the three seasons during weeks 5 and 6 in segments 5 and 6.

Perhaps the most interesting distributions are provided by the computed survivals depicted on Fig. 4a, b, and c. Graphed are the results of applying equation (15) — the cumulative probability that an egg spawned at a given time and location will survive into the post-finfold stage. That these patterns appear to vary more continuously than those in Fig. 2 and 3 is merely a reflection of the fact that survivorship is mathematically independent of the actual number of eggs spawned at a point in space and time.

It is noteworthy that processes in 1974 and 1975, with their great contrast in success, should yield highly similar patterns for observed survival. Maximal survival in both instances lies well upstream and later in the season from the centroid of spawning. It clearly appears that the spawning stock during these two seasons "underutilized" the propitious domain (weeks 6 and 7 in segments 8 and 9) for successful spawning and development. This discrepancy does not seem as apparent in 1976, when better overlap between successful eggs and high-survival regions occurred.

While these qualitative observations are interesting and informative to fisheries managers, they do not allow a direct approach to answering the fundamental question of the relative importance of environmental conditions favoring successful spawning and development versus adult spawning behavior in determining year-class success. To begin such an analysis it may be assumed that year-class success can be partitioned into a factor characterizing the environment for a given year times a factor which gauges the fitness of the spawning strategy for the same year, i.e.

$$Y = U \times F \tag{16}$$

where Y is the year-class success, U embodies the environmental conditions, and F is a measure of the fitness of the spawning behavior. U and F can be given more precise definition if we further define E as the number of eggs produced and Q as the number of post-finfold survivors produced during the year. Then, by definition

$$Y = \frac{Q}{E} \tag{17}$$

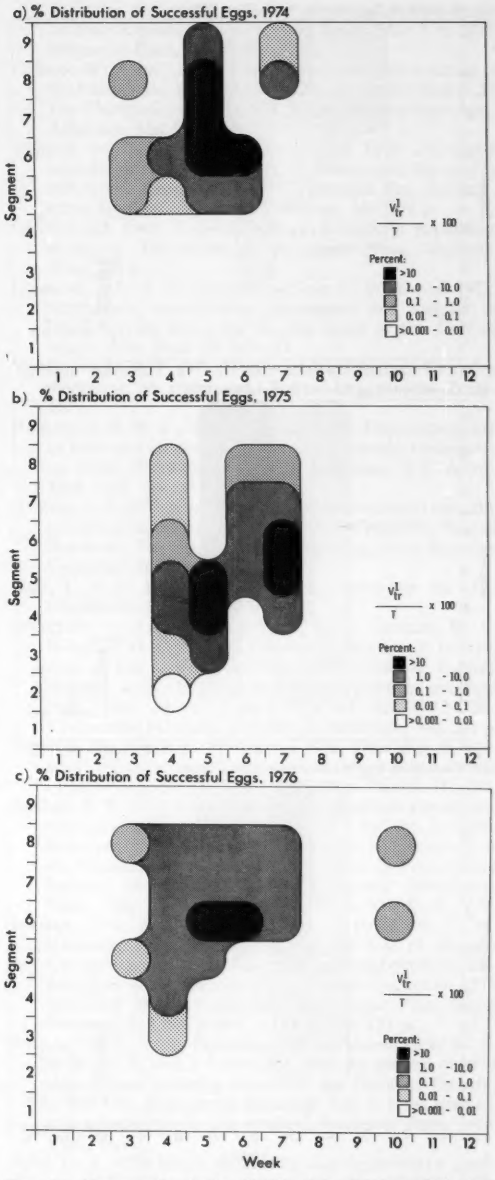

FIG. 3. Computed relative distribution of successfully developed eggs (or post-finfold larvae) during a) 1974, b) 1975, and c) 1976, according to equations (11) and (12).

Supposing that H is the hypothetical production of post-finfolds if the year-class survival had been uniformly at the greatest value observed during that par-

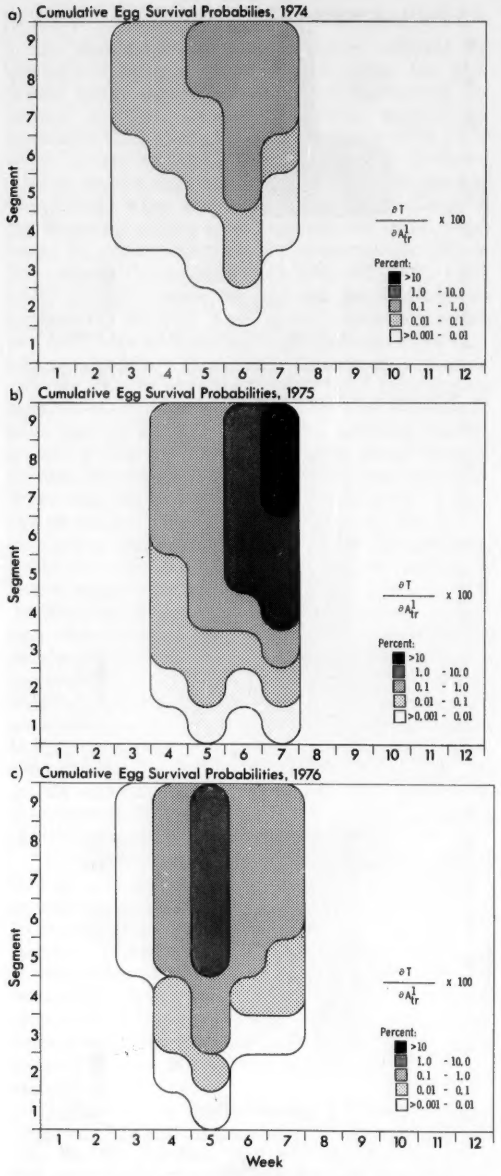

FIG. 4. Computed relative cumulative survival patterns of eggs through to the post-finfold stage during a) 1974, b) 1975, and c) 1976, according to equation (15).

ticular year, then we may write

$$(18)\quad Y = \frac{H}{E} \times \frac{Q}{H}.$$

TABLE 2. Results of success and fitness analysis.

	Year-class success Y	Maximal survival U	Spawning fitness F
1974	5.9×10^{-5}	0.0039	0.015
1975	2.1×10^{-3}	0.2100	0.010
1976	7.0×10^{-4}	0.0360	0.019

Arbitrarily choosing $U = H/E$ and $F = Q/H$ attaches straightforward meanings to these two factors. U is seen to be the ultimate year-class success possible during the year if all the eggs had been subject to the optimal conditions seen during that year. The fitness, F, becomes a measure of how well the spawning population distributed its eggs to take advantage of the best conditions available to foster egg and larval development.

The hypothetical optimal production, H, is calculated by multiplying the egg production for the year by the maximum observed survival for any time and place as calculated from equation (15).

Year-class success and its two components are displayed in Table 2. The calculated values for the fitness are interesting on two counts. First, they are quite small (<0.02). One might expect that the adult stock would have acquired migratory strategies consistent with environmental cues which would allow them better to exploit conditions favorable to ichthyoplankton survival. Secondly, the spawning fitness varies relatively little in 3 yr (2-fold), in comparison to the variation in overall year-class success (35-fold). The major element of variation in overall success is apparently determined by the independent and random environmental factors affecting optimal survival.

There are possibly two other trends seen in Table 2 which cannot be confirmed on the basis of only three seasons' data. It appears, for instance, that the calculated fitnesses are directly related to the dispersion of eggs in time and space as displayed on Fig. 2a, b, and c. Secondly, the variations in maximal survival and fitness (53-fold and 2-fold, respectively) mitigate one another slightly (success varies by 35-fold). This mitigation could easily be due to chance, to random errors in sampling, or it might be ascribed to a compensation mechanism in the spawning behavior. The type of behavioral compensation referred to here is not to be confused with the classical, density-dependent compensation in stock-recruitment relationships (Ricker 1968; Cushing 1968). Catch records and adult sex ratios show spawning stock declining over the 3 yr studied. Should behavioral compensation exist, however, it is evidently not strong enough to appreciably dampen the radical effects of environmental conditions upon larval survival.

It remains likely that maximal survival and spawning fitness are keyed by two independent variables. Apparently, year-class success and optimum-survival conditions vary radically on an annual basis due to

density-independent environmental factors. Although the spatial–temporal origin of the most successful offspring remains relatively constant from year-to-year, the strength of overall success undergoes great variation. This phenomenon seems to result from an annually constant mismatch, or "misfit," between spawning stock behavior (distributing eggs over the spawning grounds) and the larval survival patterns. There is no reason to believe that either of these phenomena is governed by factors related to each other or to conditions dependent on adult or larval population sizes.

The enigma of the very low, but relatively constant, values of the spawning fitness could be the result of a more universal ecological principle manifesting itself in the adaptation of the stock to prevailing environmental conditions. Ecological systems do not always run at maximum efficiency, and may, at times, tend only to maximize the rate of energetic storage, or production (Odum and Pinkerton 1955). It is unclear here, however, what rate process is being maximized (if any).

There are any number of other hypotheses which may explain the observed low spawning fitness. The spawning stock might be the victim of a changing environment and may not have had sufficient time to adapt behaviorally to new optimal conditions. The population may be adapted for persistence, rather than maximal production. By spawning in this inefficient manner, large pulses of production, which might cause the population to outstrip the carrying capacity of its environment, are avoided. Some spawn in the optimal regions, however, assures a residual population in bad years, thereby allowing the population to persist.

The list of speculations could go on, but would not be warranted by the present data base. A longer series of breeding seasons needs to be observed in solid quantitative fashion. Likewise, extrinsic conditions need to be closely examined to identify the factors driving the large fluctuations in year-class success.

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Oxygen Transport in Salmon Spawning Gravels

R. A. JOHNSON

Institute of Water Resources, University of Alaska, Fairbanks, AK 99701, USA

JOHNSON, R. A. 1980. Oxygen transport in salmon spawning gravels. Can. J. Fish. Aquat. Sci. 37: 155–162.

The importance of understanding transport characteristics of flow through gravel media is discussed from the viewpoint of salmonid enhancement programs. A summary of the important features of the incubation process with respect to mass transport is provided along with applicable theories describing flow through porous media. Data obtained from experiments described herein are used to assess the accuracy of existing correlations for predicting pressure drops across gravel substrates. It is found that available hydraulic relations can be used to predict flow velocity magnitudes in gravel media with an accuracy of $\pm 50\%$ over a twofold range of flow rates, providing one measurement of head loss is available at one flow rate. An adaptation of the Carman–Kozeny equation is found to be suitable for calculating the influence of fines on permeability. The importance of air entrapment on flow resistance is confirmed experimentally and modeled using available correlations. Lastly, the applications of these results for calculating oxygen transport to incubating salmon eggs and minimum water flows in hatcheries are discussed.

Key words: Salmon enhancement, oxygen transport, permeability, gravel, incubation, hatcheries

JOHNSON, R. A. 1980. Oxygen transport in salmon spawning gravels. Can. J. Fish. Aquat. Sci. 37: 155–162.

En rapport avec les programmes de mise en valeur des salmonidés, nous analysons l'importance de bien comprendre les caractéristiques de transport de l'eau à travers le gravier. Nous résumons les principales caractéristiques du processus d'incubation en relation avec le transport de masse, de même que les théories applicables décrivant le passage à travers un milieu poreux. Les données dérivées de ces expériences ont servi à évaluer la précision de corrélations existantes lorsqu'il s'agit de prédire les chutes de pression à travers des substrats de gravier. On constate que les relations hydrauliques disponibles peuvent être utilisées pour prédire les vitesses d'écoulement dans des milieux de gravier, avec précision de $\pm 50\%$ sur double gamme de vitesses d'écoulement, pourvu qu'on ait à sa disposition une mesure de perte de charge à une vitesse d'écoulement. Une adaptation de l'équation de Carman-Kozeny s'avère convenable pour le calcul de l'influence de fins matériaux sur la perméabilité. On confirme expérimentalement et modélise, utilisant les corrélations disponibles, l'importance de l'emprisonnement d'air par rapport à la résistance à l'écoulement. Enfin, nous examinons les applications de ces résultats au calcul du transport d'oxygène vers des oeufs de saumons en voie d'incubation et des débits d'eau minima dans des écloseries.

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IN both Alaska and the Pacific Northwest, extensive efforts are being devoted to salmon rehabilitation. Fundamental to these activities is that a proper environment be provided for incubating eggs. Critical to salmon egg survival is the transport of sufficient oxygen through the egg capsule to sustain the embryo's metabolic needs. For an egg with a given oxygen uptake requirement, the oxygen transfer rate will depend most critically upon the dissolved oxygen levels and interstitial flow velocities in the incubation medium of concern. The latter is also important in removing metabolic waste products.

The natural survival rate of incubating salmon eggs is normally quite low. McNeil (1976) calculates an average egg to fry survival of 8% for three species of salmon in a dozen streams in Alaska and British Columbia. Because it is possible to attain a survival rate of over 90% by using artificial incubation techniques (D. F. Alderdice, 1977 personal communication), substantial increases in fry yields can be achieved. This does not automatically translate into increased adult production. A good description of the incubation media used in hatcheries is given in Bams and Simpson (1977). It is not known how to maximize egg survival and emergence of high-quality fry as functions of flow conditions and the incubation medium (Poon 1977). The optimum economic solution is not at hand either. Moreover, the large water flow requirements of some hatcheries make a knowledge of the minimum required flow essential.

The presence of fines in the redds reduces survival

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and fry quality (Wickett 1959; Cooper 1965; Wells and McNeil 1970; Meehan and Swanston 1977). The fines cause a decrease in permeability which reduces the apparent velocity for a given pressure gradient. This phenomenon may be intensified and become a problem in terms of egg survival under low stream flow conditions because low flow promotes the setting of fines.

The purpose of this study is to quantify and delineate the applicability of relations describing flow through porous media to predict oxygen transport in salmon spawning media. These include correlations relating both mass transport and pressure drop to flow conditions and media geometries. It is hoped that those involved in the design of incubators and refurbishment of streambeds can use these results to quantify the effects of such parameters as fraction of fines present or available pressure drop on survival of eggs.

Porous Bed Theory

To assess the suitability of a given set of incubation conditions, one needs to apply theories describing flow through porous media and mass transport correlations. Once the interstitial flows are described by the former, the latter can be used to predict the rate at which oxygen is transferred to an incubating egg. These mass transport theories (Gebhart 1961; Daykin 1965) are readily available and utilize empirical correlations to calculate a dimensionless mass transfer rate, the Sherwood number

$$(1)\quad Sh \equiv \frac{kd}{\mathcal{D}}$$

where k is the mass transfer coefficient, d the diameter of an incubating egg, and $\mathcal{D}$ the diffusion coefficient for oxygen in water. For a single isolated sphere, Sh may be calculated from

$$(2)\quad Sh = 2.0 + BRe_d^{1/2}Sc^{1/3}$$

where B is an experimentally determined constant of order one, the Reynolds number $Re_d \equiv ud/\nu$, and the Schmidt number $Sc = \nu/\mathcal{D}$. Here u is the net flow velocity past the sphere and ν is the kinematic viscosity. Once k is found using equations 1 and 2, the total rate of oxygen transfer to an egg of diameter d is

$$(3)\quad \dot{m} = \pi d^2 k(C_1 - C_0)$$

Here C_0 and C_1 are the oxygen concentrations of the outer surface of the egg capsule and in the interstitial flow adjacent to the boundary layer respectively (Fig. 1).

By using equations (1–3) one can bypass the need for direct considerations of the governing boundary layer equations. The chief resistance to oxygen transport outside of the egg itself is diffusion through the boundary layer. Hence, uncertainties existing in empirical correlations for k relate to uncertainties in the boundary layer thickness and structure.

For given fluid properties and oxygen concentration drop across the boundary layer adjacent to the egg, the oxygen transfer rate can be calculated provided u is known. For an egg embedded in a porous medium, u is the interstitial velocity. In an average sense, $u = v_a/\epsilon$ where ϵ is the porosity and the apparent velocity $v_a = Q/A$. Here Q is flow rate and A is the total cross-sectional area of the porous medium perpendicular to the flow (Fig. 1). For noncapillary flow, v_a may be found from the head loss equation (Ergun 1952; Scheidegger 1960)

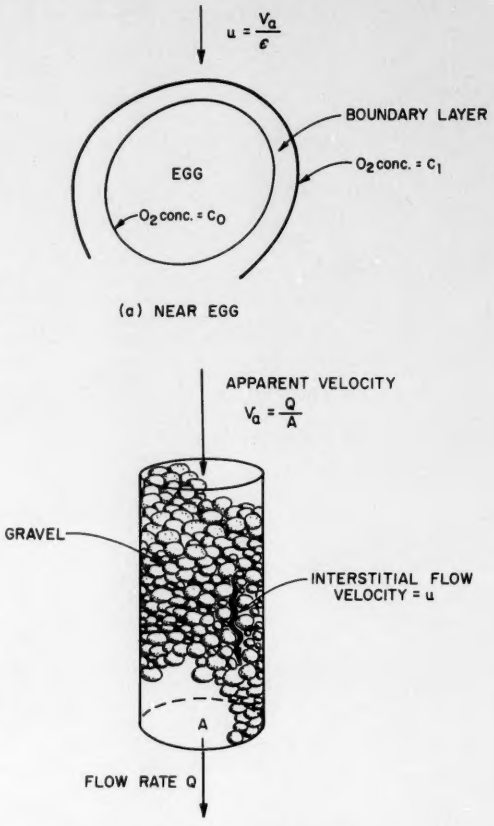

FIG. 1. Nomenclature of flow fluid around egg and in permeability column.

$$(4)\quad \frac{h}{l} = \frac{1}{K}v_a + \frac{\gamma}{(1-\epsilon)f(\epsilon)gd_e}v_a^2$$

where h/l is the head loss over a depth l, K the permeability, γ the quadratic constant, $f(\epsilon) = \epsilon^3/(1-\epsilon)^2$, g the acceleration due to gravity, and d_e an effective media particle diameter. It is defined by

$$(5)\quad d_e = (\Sigma(P_i/\psi_i d_i))^{-1}$$

Here P_i is the volume fraction and ψ_i the sphericity of a particle having a diameter d_i. The medium is assumed to be comprised of particles having diameters d_i. The

sphericity ψ_t varies between 0 and 1 and is defined as the surface area of a sphere having the same volume as the particle in question divided by the actual surface area (Fair et al. 1966).

Perhaps the most widely used formulation for predicting the permeability, K, is the Carman–Kozeny Equation (Scheidegger 1960), also developed by Fair and Hatch (1933).

$$(6)\quad K = \frac{gf(\epsilon)d_e^2}{36\kappa\nu}$$

where κ is an empirically determined permeability constant.

Ergun (1952) found it convenient to recast equation (4) in the form

$$(7)\quad \beta \equiv \frac{h}{l}\frac{(1-\epsilon)f(\epsilon)gd_e}{v_a^2} = 36\kappa\frac{1-\epsilon}{Re} + \gamma$$

where $Re = v_a d_e/\nu$ now. Here, β represents the ratio of total energy losses to kinetic energy losses. The first term on the right hand side (RHS) represents viscous effects while the second represents inertial effects. These inertial effects arise from the resistance of the fluid mass to changes in velocity. The Reynolds number provides a measure of the ratio of inertial to viscous forces. Equation (7) reverts to the conventional Darcy headloss equation when the first term on the RHS dominates. For systems comprised of various kinds of particles placed in columns having diameters $D \geq 10\ d_e$, Ergun (1952) found the data was best correlated by $\kappa = 4.17$ and $\gamma = 1.75$. Substituting these values into equation (7) leads one to conclude that inertial effects should dominate for $Re \gg Re^* = 86(1-\epsilon)$. Measured values for Re^* have varied from 0.1 to 75 (Scheidegger 1960).

Experimental Methods and Results

A series of experiments was performed to determine the accuracy of equations (4–7) for predicting flow velocities in salmon-spawning media. As shown in Fig. 2, the basic apparatus consists of a reservoir to supply a steady flow of water at a fixed head, a plexiglass permeability column, pressure taps connected to a manometer, and a receiving tank to allow flow rates to be measured. To aid in evaluating edge effects, two permeability columns with inside diameters of 8.7 and 19 cm, respectively, were used. Characteristics of the gravel media are shown in Table 1. To simulate the effects of eggs on flow resistance, glass spheres 6 mm in diameter were mixed with the gravel media for some runs.

The reduced data for the basic gravels tested appear in Table 2. The headloss vs. flow rate data for these same four basic media are plotted on Fig. 3. The coordinates for this plot are chosen to conform to the parameters appearing in equation (7). The maximum values of β, associated with low apparent velocities, correspond to the resolution of the manometer (± 0.5 mm H_2O). The smallest pressure drops occur at the lowest velocities. The minimum values correspond to a regime where inertial effects are important and the velocities and head losses are large.

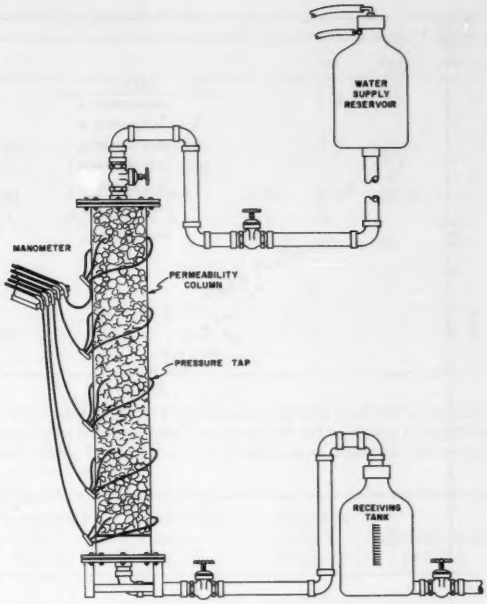

FIG. 2. Experimental apparatus.

The values of κ and γ appearing on Table 2 were obtained by fitting the data for each run to equation (4) using a least squares routine. The coefficient of variation (CV) for a given run is defined as the standard deviation for all the values of h divided by the mean value of h. The individual values of h for each run may be found from Fig. 3 and Table 2. The porosities were measured by extracting the water from each medium upon completion of a run. These values of porosity were checked by comparing with the porosity calculated from the known volume of gravel added to the column preceding a run. The difference was invariably less than 0.02. The gravel compositions by size shown on Table 1 were obtained by sieving.

To help understand the variations in bed structure caused by the randomness of the packing process, several runs were made with each gravel. Each run was preceded by carefully adding the gravel to a water-filled column in a manner that would exclude air from the pores and tamping it down. Then, water from the supply reservoir was forced through the column at velocities ranging from several hundred to several thousand cm/h. A supply reservoir was used rather than a pump to maintain steady flow during each part of a run.

For run no. 7, the gravel was added by deliberately dropping it at random into the water-filled column. This resulted in a considerable amount of air being trapped in the pores. Even though the same gravel was used as for run 6, the porosity was 23% lower for

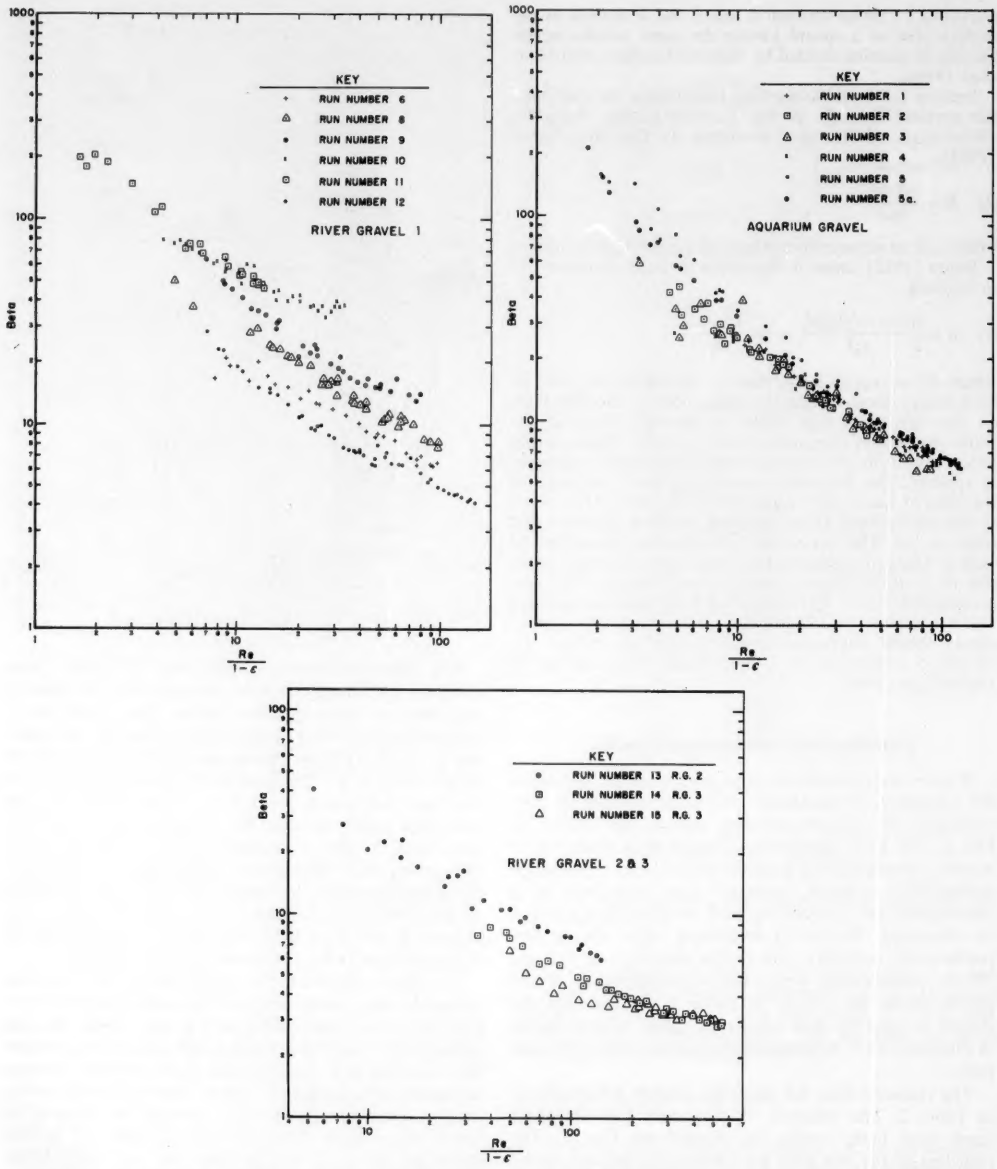

FIG. 3. Dimensionless head loss (β) vs. modified Reynolds number ($Re/(1-\epsilon)$) for aquarium gravel and river gravels 1, 2, and 3.

run 7. Here by porosity we mean fraction of the total bed volume occupied by water. From calculations involving the volumes of gravel added, we found that 14% of the pore volume was occupied by air for run 7. This partial filling of the pores with air resulted in a dramatic reduction (by a factor of 4) in permeability. However, if the data is recast in a dimensionless form as β vs. $Re/(1-\epsilon)$, it looks similar to the other data

TABLE 1. Particle size distributions for gravels tested.

										d_e	d_{max}	d_{min}
Aquarium Gravel	P_i	0.709	0.288	0.003						3.9	10	2
	d_i	6.3	3.1	2.4								
River Gravel 1	P_i	0.485	0.216	0.174	0.077	0.012	0.036	variable		5.6	13.3	0.5
	d_i	11.3	8.0	5.1	3.1	2.2	1.4	0.5 mm				
River Gravel 2	P_i	0.14	0.43	0.19	0.09	0.08	0.04	0.006	0.02	8.9	25.4	1.0
	d_i	30.0	18.4	11.3	8.0	5.1	3.1	2.2	1.4			
River Gravel 3	P_i	0.34	0.32	0.34						20	62	12
	d_i	40.2	21.9	15.9								

$d_e \equiv (\Sigma\,(P_i/\psi_i d_i))^{-1}$ is effective particle diameter, mm
P_i represents mass fraction
d_i represents geometric mean diameter from sieve analysis, mm
sphericity $\psi_i = 0.8$ for aquarium gravel and 0.9 for others.

TABLE 2. Coefficients of variation, percentage of fines (particles of 0.5 mm diameter), column and particle diameters, porosity, permeability, permeability, and quadratic coefficients, and maximum Reynolds number for aquarium gravel (runs 1–5a), river gravel 1 (runs 6–12), river gravel 2 (run 13), and river gravel 3 (runs 14–18). Tested depth, l, is 25 cm unless otherwise stated and water temperature is 23°C.

Run no.	Coefficient of variation (CV)	% Fines	Column diam, D (cm)	Particle diam, d_e (cm)	Porosity ϵ	Permeability K (cm/s)	Permeability coef. κ (Eqn. 6)	Quadratic coef. γ (Eqn. 7)	Max Reynolds no. Re_m
1	0.045	0	8.7	0.39	0.44	15.4	7.3	2.1	57
2	0.088	0	8.7	0.39	0.38	10.0	6.0	2.5	29
3	0.065	0	8.7	0.39	0.42	12.5	7.3	1.5	52
4	0.060	0	19	0.39	0.42	11.9	7.7	2.0	64
5[e]	0.036	0	19	0.39	0.43	10.0	9.0	1.9	71
5a	0.036	0	19	0.39	0.43	10.9	8.8	1.9	71
							mean = 7.7	2.0	
							CV = 0.14	0.16	
6	0.055	0	8.7	0.56	0.35	16.8	5.4	2.2	57
7[a]	0.047	0	8.7	0.56	0.27[b]	4.5	7.8	3.4	61
8	0.061	1.4	8.7	0.49	0.35	8.9	7.8	2.6	59
9	0.077	4.2	8.7	0.39	0.35	6.6	7.1	5.7	48
10	0.078	8.2	8.7	0.30	0.30	3.2	4.7	18.4	22
11	0.043	17	8.7	0.19	0.24	0.4	6.8	12.6	10
12	0.064	0	19	0.56	0.33	14.0	5.2	2.6	98
							mean = 6.4		
							CV = 0.20		
13	0.056	0	19	0.89	0.32	18.3	8.7	2.2	88
14[e]	0.031	0	19	2	0.37	178	8.3	2.4	348
15[e]	0.073	0	19	2	0.38	245	6.7	2.8	348
							mean = 7.9	2.5	
							CV = 0.13	0.12	
16[e]	0.048	0	19	1.83[c]	0.36	104	9.2	2.4	256
17[e]	0.027	0	19	1.83[c]	0.36	172	5.6	2.6	256
18[f]	0.044	0	19	1.95[d]	0.36	183	6.1	2.3	252

[a]Tested depth, l = 15 cm and gravel loaded such that air trapped in column.
[b]ϵ corresponds just to voids filled with water.
[c]5% spheres, by volume, of 6 mm diameter.
[d]2% spheres, by volume, of 6 mm diameter.
[e]Tested depth, l = 75 cm.
[f]Tested depth, l = 100 cm.

for R.G. 1. The dimensionless data is not shown in Fig. 3 to avoid unnecessary clutter.

The last 3 runs on Table 2 indicate the influence of eggs on permeability. Glass spheres were mixed with river gravel (R.G.) 3 at volume ratios of spheres to gravel up to 5%, representing a packing density up

to 280 000 spheres/m^3. This is higher than that normally used in gravel incubators (Poon 1977). The addition of the spheres did result in a lowering of the permeability.

Discussion

An analysis of the data appearing in Table 2 indicates equation (7) can be used to predict head losses or apparent velocities for the gravel media being studied. This is apparent from the small variations in κ and γ for all four basic gravels tested. The constants are to be interpreted as those which provide the best fit of our data over the range of Re tested. It is interesting to note that only for R.G. 3 was Re large enough at the higher velocities for inertial effects to dominate. It is only in this regime that the second term on the RHS of equations 4 or 7 dominates. Nevertheless, values of γ inferred from the aquarium gravel and basic R.G. 1 were similar to those inferred using R.G. 3. Even though only R.G. 3 has a size range corresponding to a good spawning gravel, the other media were tested to help establish the range of validity of our results.

Our average values for κ and γ are 7.2 and 2.2, respectively, based on the runs for which average values are indicated on Table 2. These are to be compared with best-fit values of 4.2 and 1.75, respectively, calculated by Ergun (1952). The data associated with fines are excluded here from our calculation of an average value for γ for reasons discussed later. The fact that our results are not identical to Ergun's is not surprising since there are several important distinctions between the media utilized by Ergun and those used here. First, Ergun (1952) achieved reproducible packings by initially expanding a bed with upflowing gas and then using a vibrator before allowing the media particles to settle into a stable configuration. This also caused individual particles to be oriented by the upflowing gas. Second, the data analyzed by Ergun involved gas flow through beds comprised of spheres, sand, and pulverized coke. The observed differences, therefore, may be caused by differences in mode of packing, media particles, and fluid.

The Carman–Kozeny constant κ for our beds, comprised of irregularly shaped particles, was invariably larger than 6. Evidence presented by Wyllie and Gregory (1955) indicates that κ depends on both porosity and particle shape. Using spheres, for example, κ decreased from around 7 to 5.5 as ϵ increased from 0.25 to 0.30. Different variations occur for other geometries. Rice et al. (1970) reported values for κ as high as 18 for limestone and sand particles.

Data presented by Leva et al. (1951) illustrate the importance of both the mode of packing and edge effects on bed porosity. For a variety of materials, the porosities of unpacked beds were found to be 20–50% higher than those for packed beds. The larger differences occurred with the higher ratios of average particle diameter to column diameter. For smooth spheres, the porosity differences between packed and unpacked beds remained constant over a 10-fold range of particle to column diameter. The packing was achieved by tapping on the outside of the column holding the particles for about 10 min. In our case, the data were obtained with gravel that has been packed by tamping it down with a rod. By recording data as the throughput increases, the low velocity results were not compromised by additional packing or particle reorientation possibly created by the highest velocities. However, no measurable porosity change resulted from the higher throughputs.

Data shown on Table 2 for the aquarium gravel indicate the wall to have a negligible effect on average packing for particle to column diameters, d_e/D, less than 0.04 in our experiments. This is consistent with the findings of Leva et al. (1951). Our data indicate that the porosity of aquarium gravel packed into a 10-cm diameter column to be about 5% lower than that in a 5-cm column. Hence, the wall does influence ϵ for $d_e/D > 0.08$. Data obtained by Rose and Rizk (1949) indicate the effect of the wall on head loss is less than 20% for our ranges of Re and d_e/D. Moreover, Graton and Fraser (1935) found that the haphazard nature of the packing process can cause variations in ϵ and κ even for identical media being repacked in a given container. This can cause κ to vary for a given ϵ. The data appearing in Table 2 indicate this effect to generally result in variations in ϵ of less than 10%.

The data obtained using R.G. 1 perhaps best illustrate the utility of equation (7) for predicting head losses in gravel salmon spawning gravels. In spite of various mixtures of gravels with sizes ranging from 0.6 to 13.3 mm, the mean value of κ was 6.4 with a coefficient of variation of 20%. This certainly shows that the Carmen–Kozeny Equation can be used to account for the effect of fines on bed hydraulics in the Darcy regime. This is also evident by merging of the data obtained using R.G. 1 for small values of the ordinate in Fig. 3. Hence, equation (6) can be used to estimate the effects of siltation in both natural spawning sites and hatchery incubators. These statements also apply to the case of air pockets occurring in some of the pores, providing only the wetted pores are included in the value for ϵ. For larger values of $Re/(1-\epsilon)$, the value of β depends on the fraction of fines present. This is also seen from the variation of γ in Table 2. Although some of this variation is related to the low values of Re_m, it is significant enough to make the use of equation (7) questionable at high Re when there is a large amount of fines present. Fortunately, large amounts of fines are not present in optimal spawning media so that equation (7) can then be used.

Equations (5) and (6) already account for the effect of fines on permeability, K, through their effect on effective particle diameter, d_e. The small variation in κ for R.G. 1 indicates this is sufficient providing the porosity ϵ is measured. It turns out, however, that

the effect of fines on porosity can be predicted by assuming the fines simply fill in the pores. Hence, if P is the volume fraction of fines and ϵ_0 the original porosity with no fines, the actual porosity

$$\epsilon = \epsilon_0(1 + P) - P \quad (8)$$

Equation (8) does allow the porosity data for R.G. 1 to be correlated with the volume fraction of fines. It is much simpler than a correlation presented by Camp (1964) based on data collected by Stein and Eliassen. His correlation is based on the assumption that the clogging particles form sheaths around the original media particles instead of simply filling in the pores. This sheath formation was found in the removal of iron floc by a sand filter. For the case of granular salmon-incubation media with round fine particles, equation (8) seems more appropriate. It also allowed the porosity data for spheres added to R.G. 3 to be correlated with the volume fraction of fines. Moreover, the values of κ and γ after the addition of spheres (Table 2) may be used in equations (5) and (6) to predict the effect of adding eggs on flow hydraulics in gravel media.

If gravel is shoveled into a hatchery incubation box, air pockets may be formed in some of the pores. The resulting reduction in permeability certainly impairs oxygen transfer rates. This phenomenon is probably responsible for high alevin mortalities being observed downstream of air pockets (Bailey, 1978 personal communication). It can be prevented by carefully adding the gravel slowly and by not allowing the air-saturated water supply from being warmed as it passes through the incubator or incubators. The latter will cause air to come out of solution. Localized concentrations of fines have similar effects. Of course, for a given total flow rate, a localized reduction in permeability will increase the flow rates through areas adjacent to the restricted area with a possible gain in survival. But the required pressure drop will increase. Conversely, if the pressure drop is held constant, the total flow rate will decrease as the permeability decreases.

The ultimate purpose for these empirically derived correlations is to serve as a tool in predicting mass transfer to and from incubating salmon eggs. Of crucial importance in such an application is the accuracy of this predictive tool. Our results indicate the uncertainty in apparent velocity v_a to be invariably less than ±50% for a given head loss providing one data point is available for the gravel incubation medium in question. From equation (2), one can show that an uncertainty Δv_a in v_a leads to an uncertainty ΔSh in Sh

$$\Delta Sh = \tfrac{1}{2}BSc^{1/3}Re^{-1/2}\frac{d}{\nu}\Delta v_a \quad (9)$$

It can be shown that the fractional uncertainty in Sh ($\Delta Sh/Sh$) is approximately equal to $0.5\Delta v_a/v_a$ over a wide range of practical operating conditions. This uncertainty can be added to that arising from the use of equation (2) to predict mass transfer. According to Bennett and Myers (1974), equation (2) should normally correlate mass transfer to within ±25%. Hence, the mass transfer should be able to be calculated to within ±50%, if these are the only uncertainties.

Uncertainties not addressed here include those arising from the incubating eggs being embedded in gravel and the presence of natural convection. The former introduces an additional uncertainty into the use of equation (2) while the latter may alter v_a from that predicted by equation (7) for a given head loss. Here, by natural convection, we mean fluid motion arising as a result of the transport process (Gebhart 1961). Recently, O'Brien et al. (1978) found that natural convection can be a significant driving force at some times. This convection arises from a combination of an oxygen-depleted and metabolic waste-enriched zone in the vicinity of an egg. Each of these processes causes the fluid adjacent to the egg to be denser than the ambient water and thus provides a driving force causing fluid adjacent to an egg to flow downward. In a laboratory test cell, O'Brien measured natural convective velocities of about 75 cm/h around incubating coho eggs. Hence, oxygen transport due to forced convection should be enhanced for downwelling flow. By forced convection, we mean fluid motion arising from an imposed pressure gradient.

A relevant question facing a hatchery designer is what is a safe minimum water flow rate required for the incubation boxes. Suppose mass transfer considerations indicate adequate oxygen transport rates for $Sh = 20$. Adding an uncertainty of 50% to this would obligate one to design a system such that $Sh = 30$. Use of the equations presented here would allow one to calculate a minimum approach velocity v_a for the system in question. Of course these calculations should be checked on a laboratory scale before building a full-scale hatchery. But, this approach would allow designers to estimate such variables as the influence of altering the incubation medium or changing the driving head of water on their system.

Acknowledgments

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List of Symbols

A cross-sectional area, cm²
B mass transfer constant
C_0 dissolved oxygen concentration at capsule surface, g/cm³
C_1 dissolved oxygen concentration upstream of incubating eggs, g/cm³
$\mathcal{D}$ diffusion coefficient for oxygen in water, cm²/s
D column diameter, cm
d egg diameter, cm
d_e effective particle diameter of medium, cm
d_t particle diameter, cm
g acceleration due to gravity, cm/s²
h head loss, cm
k mass transfer coefficient, cm/s
K permeability, cm/s
l characteristic length, cm
$\dot{m}$ mass transfer rate, g/s
P_t volume fraction
Q liquid flow rate, cm³/s
u interstitial velocity, cm/s
v_a apparent velocity, cm/s

GREEK SYMBOLS (dimensionless except for ν)

β ratio of total energy losses to kinetic energy losses
ϵ porosity
γ the quadratic constant
ν kinematic viscosity, cm²/s
ψ_t sphericity
κ permeability coefficient

DIMENSIONLESS PARAMETERS

$Re \equiv \frac{v_a d_e}{\nu}$ Reynolds number based on d_e

$Re_d \equiv \frac{v_a d}{\nu}$ Reynolds number based on d

$Sc \equiv \nu/D$ Schmidt number

$Sh = \frac{kd}{D}$ Sherwood number

Optimal Assay Conditions for Serum and Liver Glutamate Oxaloacetate Transaminase, Glutamate Pyruvate Transaminase, and Sorbitol Dehydrogenase from the Rainbow Trout, *Salmo gairdneri*

S. D'APOLLONIA AND P. D. ANDERSON

Biology Department, Concordia University, Montreal, Que.

D'APOLLONIA, S., AND P. D. ANDERSON. 1980. Optimal assay conditions for serum and liver glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, and sorbitol dehydrogenase from the rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 37: 163–169.

A technique often used to minitor sublethal impairment of physiological functions in fish is clinical serum enzyme analysis. However, the assay conditions used in such studies in fish systems are often those derived from mammalian studies. For some enzymes (e.g. sorbitol dehydrogenase) mammalian assay conditions are applicable to fish serum; however, for glutamate oxaloacetate transaminase and glutamate pyruvate transaminase, assay conditions must be modified for fish systems. This paper thus reports pH and substrate optima for rainbow trout SDH, GOT, and GPT. Using these optimal assay conditions, liver GOT, GPT, and SDH activities were determined to be 187, 152, and 26.6 mU/mg protein, respectively. The equivalent serum GOT, GPT, and SDH activities were 255, 17, and 17.5 mU/mL serum, respectively.

Key words: rainbow trout, serum enzyme analysis, glutamate dehydrogenase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, sorbitol dehydrogenase, liver, biochemistry

D'APOLLONIA, S., AND P. D. ANDERSON. 1980. Optimal assay conditions for serum and liver glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, and sorbitol dehydrogenase from the rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 37: 163–169.

L'analyse clinique des enzymes sériques est une méthode souvent utilisée pour suivre l'altération de fonctions physiologiques chez les poissons. Cependant, les conditions d'essais utilisés dans de telles études des systèmes ichtyologiques sont souvent les mêmes que celles dérivées d'études mammologiques. Pour certaines enzymes (e.g. sorbitol déshydrogénase), les conditions d'essais sur mammifères peuvent s'appliquer au sérum des poissons; cependant, dans le cas de la glutamate oxaloacétate transaminase et de la glutamate pyruvate transaminase, ces conditions doivent être modifiées pour les systèmes ichtyologiques. Le présent article décrit donc les pH et substrats optima pour SDH, GOT et GPT de truite arc-en-ciel. Avec ces conditions optimales d'essais, nous constatons que les activités de GOT, GPT et SDH hépatiques sont de 187, 152 et 26,6 mU/mg de protéines respectivement. Les activités équivalentes de GOT, GPT et SDH sériques sont de 255, 17 et 17,5 mU/mL de sérum respectivement.

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DYSFUNCTION in diseased organs may result in quantitative and qualitative alterations in serum enzyme activities. In clinical medicine, serum enzyme analysis has been used for decades to diagnose both the site and extent of organ injury (Bergmeyer 1974; Schmidt and Schmidt 1976). Because a large proportion of serum enzymes is derived from the liver, liver injury is especially amenable to such diagnosis. Three serum enzymes which have been commonly used as indicators of liver damage are sorbitol dehydrogenase (SDH), E.C. 1.1.1.14, glutamate pyruvate transaminase (GPT), E.C. 2.6.1.1, and glutamate oxaloacetate transaminase (GOT), E.C. 2.6.1.2 (Schmidt and Schmidt 1974).

In the field of environmental toxicology, serum and tissue enzyme analyses are becoming increasingly important for the detection of toxic effects of chemical pollutants (Eisler 1972; Jackim 1974). Two serum enzymes, frequently used by fish biologists to diagnose sublethal insult to liver by pollutants are GPT and GOT (Bell 1968; Mehrle and Bloomfield 1974; Malevski and Montgomery 1974; Racicot et al. 1975). These two transaminases, along with glutamate dehydrogenase (GlDH), play an important role in ammonia

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detoxification in freshwater teleosts (Hochachka and Somero 1973). Since nitrogen metabolism pathways differ fundamentally in fish as compared to mammals (Prosser and Brown 1973), it may be expected that the kinetic properties of the above enzymes may also differ. However, although the necessity for the use of enzyme assays procedures standardized for fish has been recognized (Bell 1968), most investigators have used assay conditions derived from mammalian studies.

Thus, one aim of this paper is to determine optimal assay conditions for rainbow trout GOT and GPT and consequently the applicability of mammalian assay conditions (especially enzyme assay kits) to the determination of fish GOT and GPT activities.

In mammalian studies, serum SDH activity has been used as a measure of liver injury (Korsrud et al. 1976; Alemu et al. 1977). Although in fish studies, liver SDH isozyme patterns have been used as population markers (Engel and Faust 1971; Khanna et al. 1975), to our knowledge, there have been no quantitative studies on either liver or serum SDH activities. Thus, a second aim of this paper is to determine rainbow trout serum and liver SDH activities.

Materials and Methods

Rainbow trout, *Salmo gairdneri*, purchased from Lac au Clair Hatchery, Lac au Clair, Quebec, were held at 11 ±1° in flow-through polyethylene tanks of 500 L capacity at a density of approximately 50 fish (200–300 mg) per tank. The flow-through rates were adjusted to maintain an oxygen saturation of 85 ±10% and adequate removal of waste products. Fish were fed daily ad libitum (Ewos Trout Chow) and acclimated a minimum of 3 wk to dechlorinated, City of Montreal Water and laboratory conditions.

All reagents were purchased from Sigma Chemical Co., Saint Louis and were of the highest purity available. The indicator enzymes LDH and MDH were treated in the following manner:

LDH — 5 mg of rabbit muscle LDH (Sigma No. L1254) were suspended in 3 mL of 10 mM sodium phosphate buffer (pH 7.4) and dialyzed three times, at 4°C, each time for 90 min, against 200 mL of 10 mM sodium phosphate buffer (pH 7.4). The volume of the dialysate was adjusted to 5 mL with additional buffer and 5 mL glycerol was added. The resulting LDH solution (240 U/mL) was stored at 0°C.

MDH — 5000 Units of beef heart MDH (Sigma No. M9004) were dialyzed three times, at 4°C, each time for 90 min, against 200 mL of 10 mM phosphate buffer (pH 7.4). An equal volume of glycerol and bovine serum albumin (to a final protein concentration of 0.5 mg/mL) were added to the dialysate. The resulting MDH solution (315 U/mL) was stored at 0°C.

Serum Preparation

Fish were anesthetized in MS222 (100 ppm) and 1 mL blood was drawn from the dorsal aorta using a 3-mL syringe rinsed with 25% sodium citrate. Blood was kept at 4°C for 2 h to allow the blood to clot prior to centrifugation for 15 min, at 3000 rpm and room temperature. The serum was removed and enzyme assays were performed.

Extract Preparation

Fish were quickly immobilized by a sharp blow on the head and the livers were excised, weighed, and immediately homogenized in cold (4°C) 0.2 M sodium phosphate buffer (pH 7.4) — 20% glycerol — 5 mM mercaptoethanol on a chilled Sorvall Ominimixer at a tissue extraction-medium ratio of 1:20. The homogenate was centrifuged in a refrigerated centrifuge (IEC B-20A) at 10 000 rpm for 15 min. The supernatant was removed and the protein concentration was determined by a modified Lowry procedure (Rabinowitz and Pricer 1962). Enzyme assays were performed immediately as enzyme activities were found to decrease upon storage (ca. 25% in 24 h at 4°C).

Enzyme Assays

Enzyme assays were performed according to the spectrophotometric methods described by Bergmeyer (1974), using a Bauch and Lomb 505 spectrophotometer fitted with a digital readout recorder. Assay volumes were adapted to give a final volume of 1 mL. The temperature was maintained at 20°C in a jacketed microcuvetted (Hellman, Cells, Inc.) through which water from a constant-temperature water bath was circulated. The rate of oxidation of NADH, as reflected by the decrease in absorbance at 340 nm, was taken as the measure of SDH, GOT, and GPT activities. The absorbance at 340 nm was recorded automatically every 1–5 s for at least 20 intervals. The least-square linear regression line of absorbance over time, computed over twenty points within the linear portion of the reaction curve, was used to determine enzyme activity. All "activities" were corrected for nonenzymatic degradation of NADH by subtracting blank values.

Glutamate dehydrogenase activity, present in liver extracts, may contribute to NADH oxidation in the GOT and GPT assays; but in mammalian assay systems, in the absence of ammonia and under optimal assay conditions for transaminase activity, this contamination is minor (Bergmeyer 1974). Bergmeyer (1974), suggested that the activity of GlDH (determined in the transaminase assays by the elimination of the amino acid substrate) be subtracted from the total activity (GlDH and transaminase activity determined in the complete assay). We followed this suggestion and subtracted the contribution of GlDH activity to the oxidation of NADH in the GOT and GPT assays.

Liver extract and serum enzyme activities are expressed as specific activity and serum activity, respectively. Student's *t*-test (Sokal and Rohlf 1969) was used to test for significance at a 95% confidence level.

$$\text{Specific activity (mU)} = \frac{\text{nmol NADH oxidized}}{\text{min}^{-1} \cdot \text{mg protein}^{-1}}.$$

$$\text{Serum activity (mU)} = \frac{\text{nmol NADH oxidized}}{\text{min}^{-1}\text{m} \cdot \text{mL serum}^{-1}}.$$

Results and Discussion

Glutamate Oxaloacetate Transaminase and Glutamate Transaminase

Studies were undertaken to determine the optimal

TABLE 1. Effect of sonication on G1DH, GOT, and GPT activities in crude liver extracts (assay conditions as in Table 4). (Each value represents eight replicates of a pooled serum sample from three fish). (*determined under optimal GOT conditions; **determined under optimal GPT conditions.)

Enzyme assay	Specific activity mU/mg protein ±SD	
	Sonication	No sonication
G1DH*	59±4	25±2
GOT	172±13	248±17
G1DH**	37±1	8±2
GPT	198±6	228±7

assay conditions for the determination of the liver transaminases, GOT and GPT, extracted from rainbow trout liver and serum.

Since the transaminases are both cytoplasmic and mitochondrial enzymes, quantitative extraction of GOT and GPT has usually included a sonication step. However, our preliminary experiments (Table 1) showed that although sonication increased the G1DH activity (determined under GOT and GPT optimal assay conditions), the GOT and GPT activities of the crude extract were significantly decreased. Thus liver extracts were not subsequently sonicated. The decreases in GOT and GPT activities may be due to competition by G1DH for NADH and α keto gluterate in the complete transaminase assays. The alternative solution of changing the concentration of the transaminase substrates in accordance with the G1DH activity in the extract was not attempted in order to minimize variables.

Glutamate dehydrogenase present in the crude liver extract or in serum, in the presence of ammonia will compete with GOT and GPT for NADH and α keto glutarate (Fahein et al. 1977). As exogenous ammonia is often present in the purchased indicator enzymes and enzyme kits, we investigated the influence of ammonia on G1DH, GOT, and GPT activities. Table 2 demonstrates that 100 mM ammonium sulphate significantly increases the contribution of G1DH activity to NADH oxidation in the GOT and GPT assays without significantly altering transaminase activities. In the GPT assay, in the presence of 100 mM ammonium sulphate 49% of the NADH that is oxidized is oxidized by G1DH, *not* GPT. Even in the absence of added exogenous ammonium ion, a significant amount of the NADH that is oxidized in both the GOT and GPT assays is oxidized by G1DH rather than by the assayed transaminase.

TABLE 2. Effect of 100 m*M* ammonium sulphate on NADH oxidation by G1DH, GOT, and GPT (assay conditions as in Table 4). (Each value represents eight replications of a pooled serum sample from three fish).

Assay conditions	Specific activity (mU/mg protein) ±SD		
	Total	G1DH contribution	Transaminase contribution
GOT assay			
(+NH_3)	367±28	63±5	304±23
(−NH_3)	328±25	42±3	286±21
GPT assay			
(+NH_3)	258±17	85±2	173±15
(−NH_3)	205±14	31±3	164±14

TABLE 3. Assay conditions used for rainbow trout liver transaminases and SDH

Assay condition	GOT assay	GPT assay	SDH assay
pH	7.2	7.2	7
Phosphate	80 mM	80 mM	80 mM
NADH	0.125 mM	0.100 mM	0.150 mM
DL alanine	—	100 mM	—
DL aspartate	100 mM	—	—
MDH content	6.3 U	—	—
LDH content	—	4.8 U	—
α keto glutarate	2 mM	2 mM	—
Fructose	—	—	400 mM
Serum volume	0.02 mL	0.08 mL	0.02 mL
Liver extract (μg protein)	0.2	0.7	3.0

Thus in rainbow trout, unlike in mammalian systems G1DH interferes with the transaminase assays. This is especially intensified in the presence of ammonia. Thus rigorous attention must be expended on removing exogenous ammonia from the indicator enzyme preparations. The concurrence of G1DH with the transaminases in liver extracts is not merely a technical problem but may have physiological significance. The activity of G1DH, an allosteric enzyme (Hochachka and Somero 1973), relative to that of the transaminases may determine the direction of amino acid metabolism (catabolism versus anabolism).

An additional problem is posed when assaying GOT and GPT in fish exposed to certain hepatotoxicants in that blood ammonia levels were observed to increase markedly upon treatment with dieldrin or carbon tetrachloride (d'Apollonia and Anderson 1977). This observation suggests that in studies on the effect of hepatotoxicants on fish serum transaminase levels, the increases in serum GOT and GPT may be a reflection of changes in serum ammonia.

Figures 1 and 2 represent the pH, NADH, α keto glutarate, amino acids, and extract volume optima for GOT and GPT determinations. The assay conditions (Table 3) which were subsequently used differ from mammalian assay systems chiefly in the lower α keto glutarate and amino acids concentrations. The precision of the GOT and GPT assays are 7 and 6.7%, respectively, determined according to Buttner et al. (1974). Thus these assays are both quick (8/h, including correction for G1DH) and precise.

SORBITOL DEHYDROGENASE

Studies were also undertaken to determine the

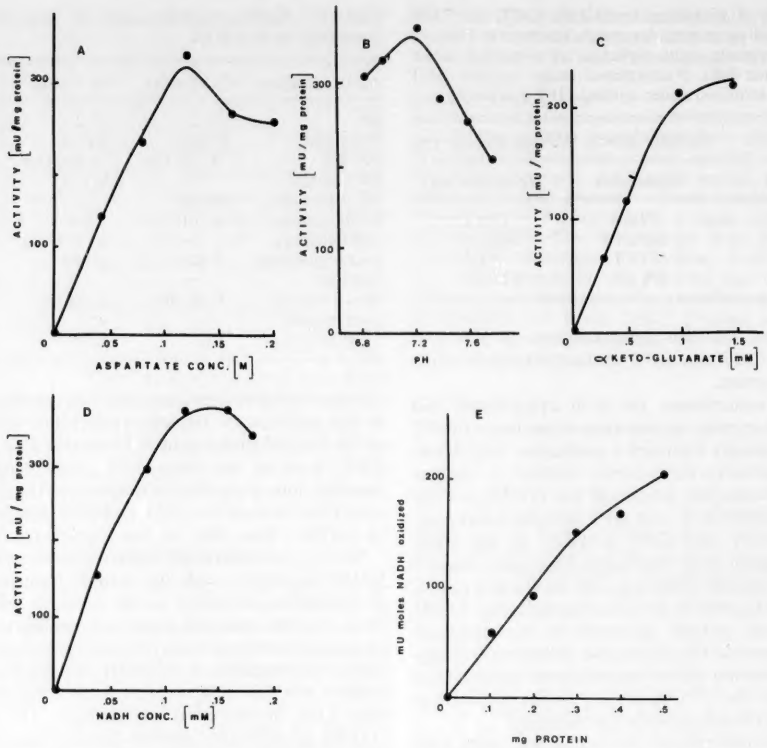

FIG. 1. Effect of aspartate concentration (A), pH (B), α keto glutarate concentration (C), NADH concentration (D), and extract protein concentration (E) on liver glutamate oxaloacetate transaminase of rainbow trout. Unless noted, all assay conditions as in Table 3.

optimal assay conditions for liver SDH extracted from rainbow trout. Figure 3 illustrates the pH, fructose, NADH, and extract volume optima for SDH.

The SDH assay conditions utilized in all subsequent enzyme determinations are presented in Table 3. These conditions are very similar to those used in mammalian studies. The precision of the SDH assay (under the assay conditions presented in Table 3) was found to be 3.1%, determined according to Buttner et al. (1974).

SERUM AND LIVER GOT, GPT, AND SDH ACTIVITIES

The rainbow trout liver and serum GOT, GPT, and SDH activities are presented in Table 4. Enzyme activities obtained from different studies are not comparable when the assay conditions vary. For example, in two similar studies on rainbow trout transaminases, one group used assay kits containing ammonium sulfate (Gaudet et al. 1975) while the other group used suboptimal assay conditions, but corrected for G1DH contamination (Pfeifer et al. 1977). As is expected, the transaminase activities, especially the GPT activity in the former study are significantly higher than those in either this study or the Pfeifer et al. (1977) study. On

TABLE 4. Levels of SDH, GOT, and GPT in rainbow trout liver and serum.

	Enzyme activity (mU) ±SD	
	Liver	Serum
GOT	187±12 (10)[a]	255±29 (20)[a]
GPT	152±12(10)	17±4 (20)
SDH[b]	26.6±1(10)	17.5±4.5 (20)
GOT[c]	130±6 (13)	113±27 (7)
GOT[b]	300 (2)	259±36 (10)
GPT[c]	295±26 (13)	9.3±1 (15)
GPT	350 (2)	54±8 (17)

[a]Numbers in brackets refer to number of samples.
[b]Pfeifer et al. (1977)
[c]Gaudet et al. (1975)

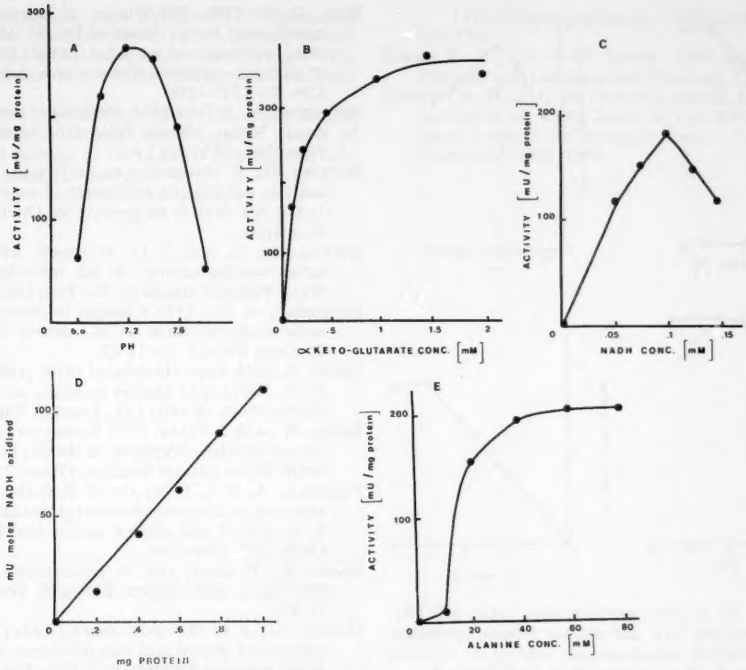

FIG. 2. Effect of pH (A), α keto glutarate concentration (B), NADH concentration (C), extract protein concentration (D), and alanine concentration (E) on liver glutamate pyruvate transaminase of rainbow trout. Unless noted, all assay conditions as in Table 3.

the other hand, the transaminase activities, with the exception of liver GPT, determined in this study are significantly higher than those reported by Pfeifer et al. (1977).

We attempted to normalize for the different assay conditions used in the above mentioned studies by comparing both the ratios of liver to serum transaminase activities and the ratios of GOT to GPT. However, these ratios also vary widely (Table 5). We perceive two reasons for this discrepancy. Firstly, the influence of ammonia is greater for GPT than for GOT. Secondly, at suboptimal GOT and GPT assay conditions, liver G1DH competes effectively for α keto glutarate and NADH, thus the transaminase activities are lower.

Rainbow trout liver and serum SDH activities were 26.6 mU/mg protein and 17.5 mU/mL serum, respectively. These values are several fold higher than SDH activities reported for a number of mammalian species. Cattle (Frahm et al. 1977) and rat (Egolinskii 1976) liver SDH activities have been reported to be 4.28 and 11.8 mU/mg protein, respectively, while cattle (Frahm et al. 1977), rat (Kosgrud et al. 1976), and sheep (Alemu et al. 1977) serum SDH activities have been reported to be 4.4, 2.2, and 7 mU/mL serum, respectively. The serum SDH activity in this report represents the serum SDH activity in female fish. Serum SDH activity in male fish is significantly higher (d'Apollonia and Anderson unpublished data).

The higher serum SDH activity in rainbow trout suggests that either there is an additional source of serum SDH (other than liver or testis) or that the turn-over

TABLE 5. Comparison of rainbow trout liver and serum ratios in various studies.

Ratio	This study	Pfeifer et al. (1977) study	Gaudet et al. (1975) study
Liver $\frac{GOT}{GPT}$	1.2	0.5	0.85
Serum $\frac{GOT}{GPT}$	12.0	12.0	5.0
$\frac{Liver}{Serum}$ GOT	0.75	0.11	1.2
$\frac{Liver}{Serum}$ GPT	9.0	3.0	7.0

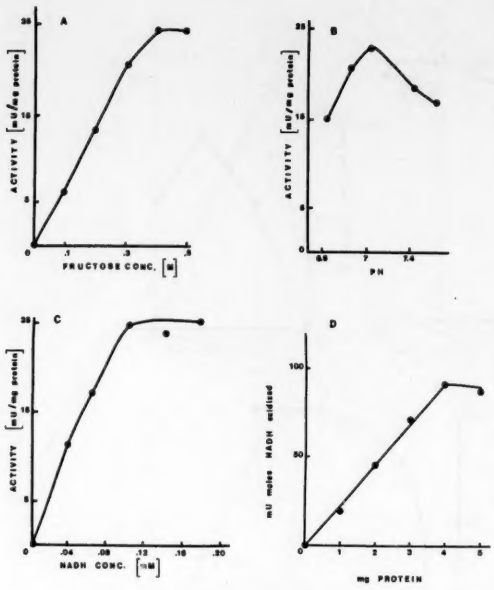

FIG. 3. Effect of fructose concentration (A), pH (B), NADH concentration (C), and extract protein concentration (D) on sorbitol dehydrogenase activity of rainbow trout. Unless noted, all assay conditions as in Table 3.

of serum enzymes is slower in rainbow trout (at 10°C) than in mammals (at 37°C). The latter possibility is more probable as serum GOT and GPT are also higher in rainbow trout than in mammals (Zimmerman et al. 1969).

In conclusion, serum enzyme analyses offer many advantages as a tool for the quantitation of liver dysfunction. These are: the small sample volume, the speed of analysis, and the full recovery of the fish; thus allowing for the possibility or both serial sampling and field sampling. Although there have been many suggestions (e.g. Eisler 1968) that clinical blood tests be used as indicators of toxic hazard, one should not assume, based on these studies, that mammalian test kits or assay conditions are directly applicable to fish studies.

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Effects of Dietary Ascorbic Acid on Chronic Lead Toxicity to Young Rainbow Trout (*Salmo gairdneri*)

P. V. HODSON

Department of Fisheries and Oceans, Great Lakes Biolimnology Laboratory, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6

J. W. HILTON

Department of Nutrition, College of Biological Sciences, University of Guelph, Guelph, Ont. N1G 2W1

B. R. BLUNT

Department of Fisheries and Oceans, Great Lakes Biolimnology Laboratory, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6

AND S. J. SLINGER

Department of Nutrition, College of Biological Sciences, University of Guelph, Guelph, Ont. N1G 2W1

HODSON, P. V., J. W. HILTON, B. R. BLUNT, AND S. J. SLINGER. 1980. Effects of dietary ascorbic acid on chronic lead toxicity to young rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 37: 170–176.

Newly hatched rainbow trout (*Salmo gairdneri*) exposed to waterborne lead and receiving diets deficient in, or supplemented with, ascorbic acid, developed classical symptoms of lead toxicity and ascorbic acid deficiency. Those exposed to lead showed elevated blood lead concentrations, inhibition of erythrocyte δ-amino levulinic acid dehydratase, darkening of the tail regions and spinal curvatures. Those receiving ascorbate deficient diets showed depletion of carcass, liver, kidney, and brain ascorbic acid concentrations plus spinal curvatures. Fish subjected to both treatments simultaneously showed no evidence of an enhancement of ascorbate deficiency symptoms by exposure to lead or enhancement or reduction of lead toxicity symptoms by ascorbate deficient or supplemented diets, respectively. These results demonstrate that, although aspects of lead toxicity in fish resemble ascorbic acid deficiency, there is no metabolic interaction between the two factors.

Key words: pollutants, toxicity, interaction, lead, vitamin deficiencies, Salmonidae, rainbow trout

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Des truites arc-en-ciel (*Salmo gairdneri*) nouvellement écloses exposées à du plomb contenu dans l'eau et recevant des régimes déficients en acide ascorbique ou avec supplément de cet acide développent les symptômes classiques de toxicité par le plomb et de déficience d'acide ascorbique. Les truites exposées au plomb montrent une plus forte concentration de plomb sanguin, inhibition de la δ-aminolevulinique acide déshydratase des érythrocytes, assombrissement de la région caudale et courbatures de l'épine dorsale. Les truites nourries à des régimes pauvres en ascorbates montrent un épuisement des concentrations d'acide ascorbique dans le squelette, foie, rein et cerveau, en plus de courbatures de l'épine dorsale. Les poissons soumis simultanément aux deux traitements ne montrent pas de signe d'aggravation des symptômes de déficience d'ascorbates causée par exposition au plomb, ou augmentation ou réduction des symptômes de toxicité par le plomb causée par des régimes dans lesquels les ascorbates sont déficients ou ont été ajoutés respectivement. Ces résultats démontrent que, bien que les aspects de la toxicité par le plomb chez les poissons ressemblent à une déficience d'acide ascorbique, il n'y a pas d'interaction métabolique entre les deux facteurs.

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LEAD is a peripheral neural toxicant that impairs learning behavior in both mammals and fish (Chisholm 1971; Weir and Hine 1970). Low concentrations of waterborne lead cause additional symptoms in fish characteristic of neuromuscular damage. These include: blackening of the skin in the caudal region (black tails), lordosis and scoliosis, and muscle tremors (Davies et al. 1976; Holcombe et al. 1976; Hodson et al. 1978). These symptoms are followed by atrophy of the tail region,

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secondary fungal infections, and death. All of the above symptoms increase in severity and frequency in a test population as the lead concentration and exposure time are increased.

The above symptoms are also characteristic of some disease states and nutritional deficiencies. Skin darkening is caused by any factor affecting neural or hormonal control of melanin dispersion (Roberts 1975). Whirling disease, resulting from infection by *Myxosoma cerebralis*, causes skin darkening due to nerve injury following spinal flexure (Roberts 1975; Davies et al. 1976). Ascorbic acid deficiency causes spinal curvature through impaired collagen formation (Halver et al. 1969) and tryptophan deficiency causes spinal curvature through possible reduction of serotonin synthesis (Kloppel and Post 1975).

Lead may interact with any of the above factors to produce the observed symptoms or it may cause neuromuscular damage by an unrelated mechanism. Davies et al. (1976) demonstrated that *Myxosoma cerebralis* and *Ichthyosporidium hoferi* were not causative factors in lead-induced injury to fish, but the other three possibilities remain unexplored. This experiment assessed ascorbate–lead interactions by comparing the toxicity of lead to trout on an ascorbate deficient diet with toxicity to trout on ascorbate supplemented diets.

Materials and Methods

Design

Newly hatched rainbow trout were exposed to nominal waterborne lead concentrations of 0 (control), 32 and 100 μg/L at 15°C. They were fed a commercial trout starter until the average size was 1.0 g. Subsequently fish at each lead concentration were fed experimental diets containing nominal ascorbic acid concentrations of 0 (deficient), 100 (control), and 1000 (supplemented) mg/kg of dry feed. The 9 lead × ascorbate treatment combinations were replicated three times for a total of 27 tanks with three extra control tanks (0 μg/L lead, 100 μg/kg ascorbate) added to ensure sufficient controls should an accident occur. Records were kept of growth, mortality, incidence of black tails and incidence of spinal curvatures, as well as tissue ascorbate levels, over an exposure period of 16 wk. Four fish per tank were sampled for determination of tissue ascorbate concentrations, blood lead concentrations, erythrocyte δ-amino levulinic acid dehydratase (ALA-D) activity, blood iron concentration, red blood cell count and condition factor according to the experimental schedule shown in Fig. 1.

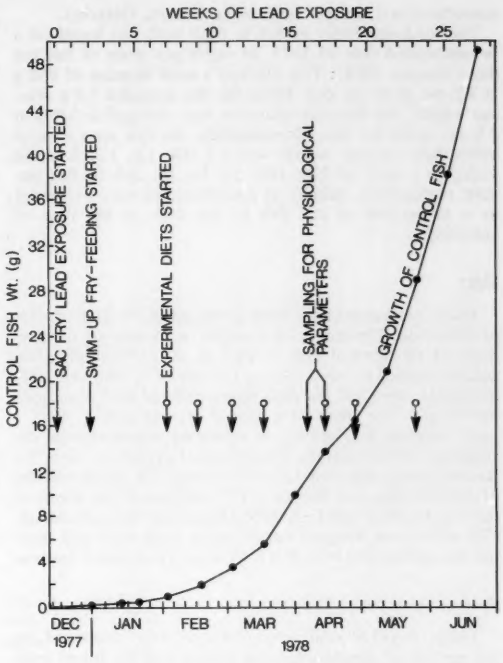

FIG. 1. Sampling schedule for this experiment. The growth curve indicates average fish weight at each sampling and the open circles indicate sampling dates for tissue ascorbate analyses.

Water

The dilution water was a dechlorinated municipal supply drawn from Lake Ontario (Hodson et al. 1977). The means and standard deviations of daily measurements of temperature and weekly measurements of dissolved oxygen, pH, conductivity, and hardness were 14.9 ± 0.9°C, 8.2 ± 1.2 mg/L, 7.86 ± 0.19, 273 ± 8 μmhos/cm (μS/cm) and 135 ± 2.5 mg/L as $CaCO_3$, respectively. Lead was added to the water by a doser and diluter adopted from Hodson (1979) and Mount and Brungs (1967), respectively. The diluter delivered 6.1 L of water per concentration every 2 min. This was split to give 340 mL/min to each of nine tanks containing 20 L of water. Water for the three extra control tanks came from one extra cell in the diluter. Waterborne lead was measured daily by atomic absorption spectrophotometry (IWD 1974) in samples from one tank at each nominal lead concentration. Different tanks were sampled each day.

Fish

Rainbow trout eggs were obtained from Norfolk Trout Fisheries, Simcoe, Ontario, within 3 h of fertilization. They were incubated at 11°C for 26–36 d to hatch. After hatching, 300 sac fry were transferred to each experimental tank and the exposure to lead started. The fry averaged 0.12 g at swim-up (when active swimming and feeding began) and were fed a commercial trout starter because of its high lipid content and finely ground state. This starter contained 200–250 mg/kg ascorbate. The fish were weighed weekly and, when the average wet weight was 1.0 g, feeding of the experimental diets was initiated. At this time, the lead exposure time from hatching was 7 wk. Subsequently, all fish from each tank were weighed biweekly as a group. At intermediate weeks, only subsamples of fish were weighed. Ration levels and pellet sizes were adjusted for fish size according to the recommendations of a commercial feed

manufacturer (Martins Feed Mills, Elmira, Ontario).

The loading density of fish in each tank was based on a recommended flow of 2–3 L of water per gram of fish per day (Sprague 1969). This allowed a total biomass of 160 g at 3 L per gram per day. When the fish exceeded 3.2 g average weight, the biomass limitation was changed to 240 g at 2 L per gram per day. Consequently, the fish were thinned when their average weight was 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 g to a total of 200, 100, 50, 30, 20, and 10 fish per tank, respectively. Results of measurements were expressed as a proportion of the fish in the tank at the time of sampling.

Diet

The experimental diets were prepared in the Department of Nutrition, University of Guelph, according to the formula of the control diet of Cho et al. (1976). Ascorbic acid was added to these diets at the rate of 0, 100, and 1000 mg/kg dry feed and the diets steam pelleted in a laboratory pellet mill. The diets were stored in a freezer (−20°C) until required for feeding, to eliminate severe storage destruction of the vitamin. Periodic analysis of the diets for postprocessing concentration of ascorbic acid by the method of Aeschbacker and Brown (1972) indicated that losses of the vitamin were less than 15% throughout the experiment. Fish diets were weighed out daily for each tank and feeding was spread out over 4–8 h by small mechanical feeders.

Sample Analyses and Statistics

Tissue ascorbic acid concentrations were measured by the method of Aeschbacker and Brown (1972). Blood lead, erythrocyte ALA-D activity, blood iron, red blood cell counts, and hematocrit were measured according to Hodson et al. (1977, 1978). Condition factor was calculated from length (L; cm) and weight (W; g) according to the formula:

$$K = \frac{W \times 10^2}{L^3}$$

All results were analyzed by factorial analyses of variance (Steel and Torrie 1960) and treatments were compared at the 95% confidence level by partitioning the treatment sums of squares.

Results

The means and standard deviations of 140 daily measurements of waterborne lead were 2.9 ± 1.9, 28 ± 12, and 87 ± 36 μg/L at nominal concentrations of 0 (control), 32, and 100 μg/L, respectively. Mortality of fish was not affected by waterborne lead or dietary ascorbate when they were less than 20 g (less than 20 wk posthatching). At average weights above 20 g, there were 10 fish or less per tank and mortality appeared to increase with increasing lead and increasing dietary ascorbate. However, the mortality in any treatment was always 10% or less of total fish exposed. Due to the limited number of fish and low incidence of mortalities, no statistical conclusions were made.

Growth was affected slightly by both lead treatments and dietary ascorbic acid (Fig. 2). Trout fed a deficient diet grew at essentially the same rate as those fed the control diet, while those fed the supplemented diet were

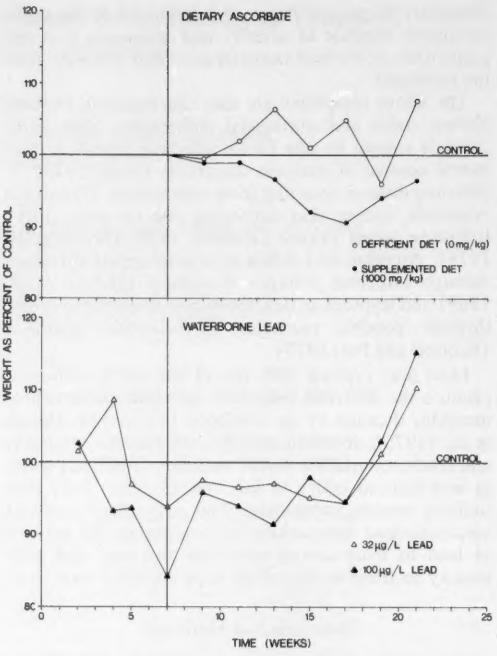

FIG. 2. Relative weights of experimental fish. The upper figure demonstrates ascorbic acid effects while the lower figure demonstrates lead effects. At any one time, the deviations from control values were not statistically significant. There were no lead–ascorbate interactions.

slightly (less than 10%) but consistently smaller than controls. Lead treated fish were also smaller than controls and those at 100 μg/L were smallest (Fig. 2). Except for one sampling time, the weight of lead-treated fish was within 10% of control weights. No lead–ascorbate interactions on fish weight were observed. When the average weight exceeded 20 g (after 20 wk), weight results became unreliable due to sampling bias caused by less than 10 fish per tank.

The incidence of black tails was directly related to waterborne lead exposure. The incidence increased with time (Fig. 3) so that after 20 wk on the experimental diet (27 wk lead exposure), all fish at 100 μg/L and 9% of fish at 32 μg/L lead exhibited black tails, while control fish exhibited no black tails. Dietary ascorbate deficiences and supplementation did not affect the incidence of black tails either directly or by interaction with lead exposure.

Symptoms of spinal curvatures did not appear until 12 wk from the start of the test diet. The incidence was always less than 18% for any treatment and increased with increasing concentration of waterborne lead and decreasing concentration of dietary ascorbate. These symptoms first appeared when the fish averaged

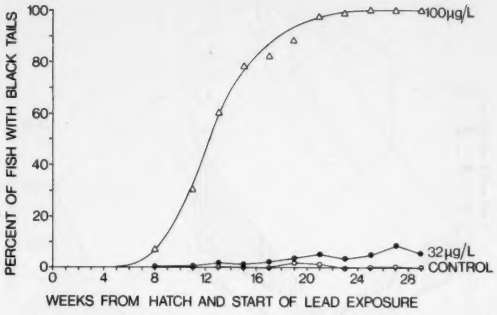

FIG. 3. The incidence of black tails of lead-exposed fish during the experiment.

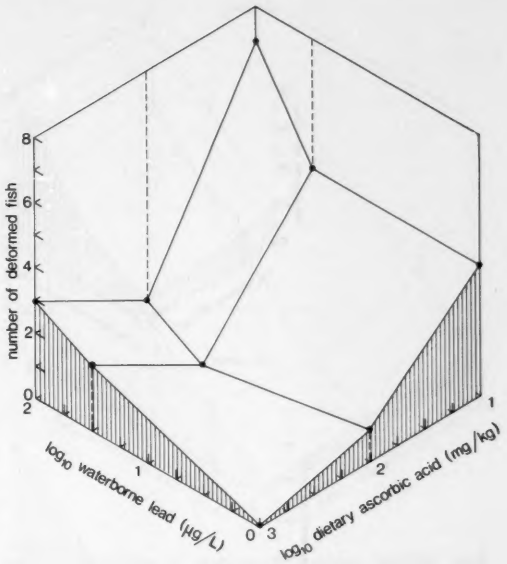

FIG. 4. Variation of the total number of fish observed with spinal curvatures after 22 wk on the experimental diets.

16 g and there were 14 or fewer fish per tank. Since some of the affected fish were removed by sampling and thinning, the incidence often decreased or increased dramatically. The results were therefore expressed as the total number of affected fish observed throughout the experiment at each treatment (Fig. 4) although there were too few data for statistical analysis.

Tissue ascorbic acid concentrations strongly reflected dietary ascorbate but not lead exposure (Fig. 5). The deficient diet caused decreasing ascorbate concentrations with time in all tissues examined, although part of this decrease was attributable to an overall decrease as the fish grew larger. Carcass ascorbate in fish on the deficient diet averaged 79% of controls whereas carcass ascorbate of fish on the supplemented diet was increased by 30% over controls. Ascorbate levels in liver, kidney, and brain of fish on the deficient diet averaged 25, 35, and 35%, respectively, of controls, whereas in fish on the supplemented diet, the levels increased by 115, 54, and 45%, respectively, over control values. Lead by contrast had only a negligible effect on tissue ascorbate. In only one case did waterborne lead increase tissue ascorbate significantly. Analysis of variance indicated that carcass ascorbate after 4 wk on the test diets was significantly elevated at the 95% confidence level at both lead concentrations and the maximum elevation was 30% above control levels. Overall, high lead exposure increased ascorbate content of carcass, liver, and head kidney by 5, 20, and 6%, respectively, but the effect was not consistent enough to be significant. Brain ascorbate was not affected by lead exposure. There were no significant lead–ascorbate interactions on tissue ascorbate identified by analyses of variance.

Blood lead concentrations varied directly with waterborne lead and were totally unaffected by dietary ascorbate or interactions between dietary ascorbate and waterborne lead (Fig. 6). Average blood lead concentrations of fish exposed to 100 µg/L waterborne lead increased by a factor of about 5 over control blood lead. Erythrocyte ALA-D activities (as nano Moles porphobilinogen/mL Red Blood Cells/h — nM PBG/mL RBCs/h) were also related only to waterborne lead. The ALA-D activity of fish at 100 µg/L was about 30–50% of that of control fish and was unaffected by the level of dietary ascorbate (Fig. 7).

The condition factor (K) plus other hematological parameters, including hematocrit, blood iron, red blood cell counts, and red blood cell volumes were unaffected by either dietary ascorbate, waterborne lead or the interaction between these treatments. The mean values plus or minus pooled standard deviations for these parameters in control fish were 1.801 ± 0.201, $30.9 \pm 2.3\%$, 170 ± 45 µg Fe/mL whole blood, 971000 ± 45000 RBCs/mm^3, and $0.322 \pm 0.057 \times 10^{-6}$ µL, respectively.

Discussion

Symptoms of both chronic lead toxicity and ascorbic acid deficiency appeared in rainbow trout during the course of this study. As lead exposure increased with time and concentration, the trout exhibited an increasing incidence of black tails (Fig. 3) followed by spinal deformities (Fig. 4). These signs coupled with a significantly higher blood lead level (Fig. 6) and a decreased activity of erythrocyte ALA-D (Fig. 6) were indicative of lead toxicity in these trout (Davies et al. 1976; Holcombe et al. 1976; Hodson et al. 1978). Similarly, the appearance of lordosis and scoliosis (Fig. 4) and the significant reduction in tissue ascorbate levels

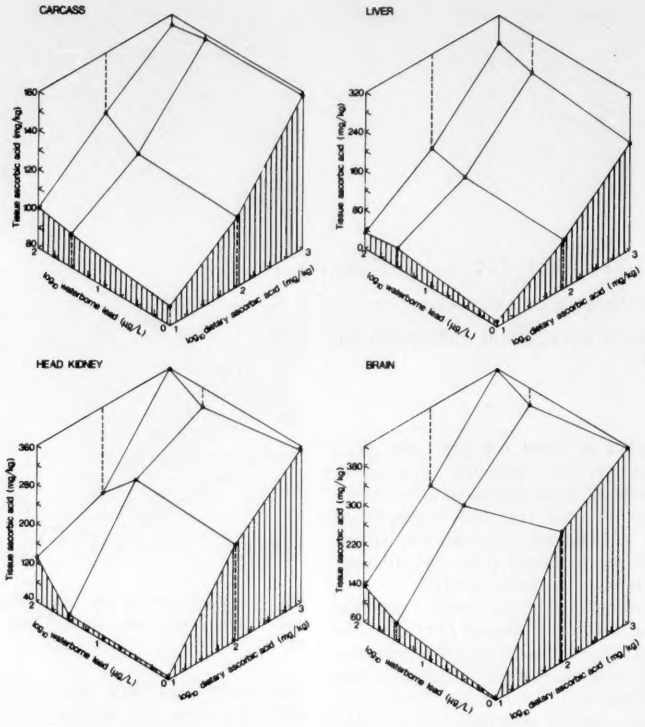

FIG. 5. Variation of tissue ascorbic acid concentrations in the carcass, liver, head kidney, and brain with nominal waterborne lead concentration and dietary ascorbic acid level. Each point represents the grand average for that treatment over three sample times. The standard deviations based on pooled error variances for carcass, liver, head kidney, and brain were 30.6, 49.5, 97.5, and 87.7 mg/kg, respectively. Only dietary ascorbic acid significantly affected tissue ascorbic acid concentrations.

(Fig. 5) were indicative of ascorbic acid deficiency (Hilton et al. 1977, 1978).

The appearance of signs of ascorbate deficiency and lead toxicity permitted an evaluation of possible interactions between ascorbic acid and lead in rainbow trout. First, lead could have enhanced or induced ascorbate deficiency in fish by increasing the utilization of ascorbate to either excrete or store lead or repair damage created by lead. If this were the case, tissue ascorbate levels would have decreased as lead exposure increased and ascorbate supplementation would have counteracted the lead effects. Neither result was observed. Secondly, lead could have interfered directly in the metabolism of ascorbic acid through inhibition of a reaction involving ascorbate. In this case, tissue ascorbate would have increased with lead exposure due to decreased utilization. If the inhibition was competitive, dietary supplementation of ascorbate would have reduced the severity and incidence of lead toxicity symptoms. Again, neither result was observed. The increase in the incidence of spinal deformities in the trout treated with both high lead levels and low dietary ascorbic acid suggests a synergistic response. However, the small number of affected fish and the appearance of isolated scoliotic fish in other treatment groups (Fig. 4) suggests that the interaction is most likely additive, with the discrepancy in addition being due to random error. It is concluded, therefore, that the etiology of lead toxicity in trout is not directly related to the metabolism or function of ascorbic acid.

Previous studies with mammals provide evidence both for and against a beneficial effect of ascorbate treatment in lead toxicity. Pillemer et al. (1940) showed that although lead toxicity was greater in ascorbic acid deficient guinea pigs compared to those receiving adequate ascorbate, the vitamin only prevented the onset

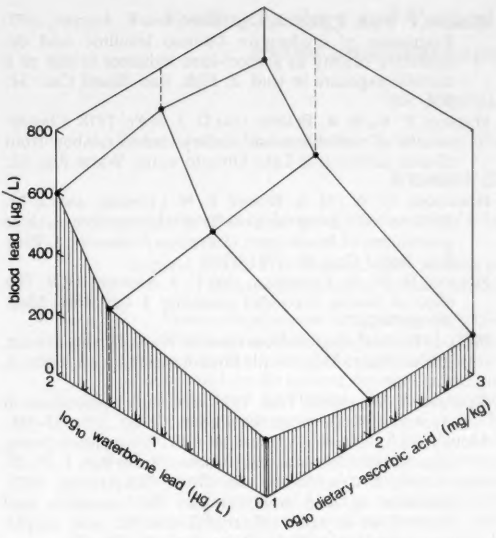

FIG. 6. Variation of blood lead concentrations with nominal waterborne lead concentrations and dietary ascorbic acid level. The standard deviation based on the pooled error variance was 98 µg/L. Only waterborne lead had a statistically significant effect on blood lead.

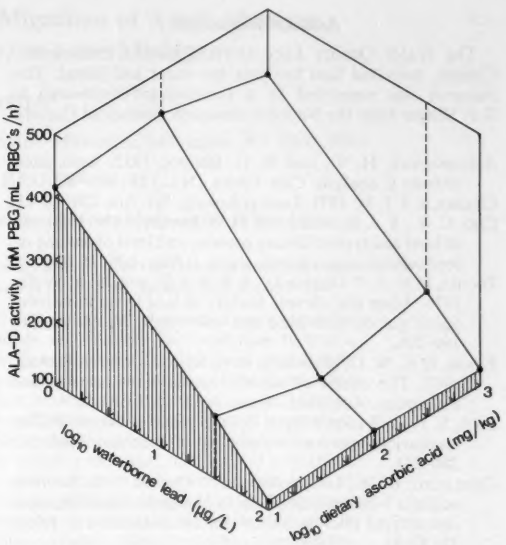

FIG. 7. Variation of erythrocyte δ-amino levulinic acid dehydratase activity with nominal waterborne lead concentration and dietary ascorbic acid level. The standard deviation based on the pooled error variance was 82 nM PBG/mL RBCs/h. Only waterborne lead had a statistically significant effect on ALA-D activity.

of neurotoxicity and not lead effects on growth, anemia and red blood cell stippling. No evidence was found for a lead effect on ascorbate utilization or metabolism. Lead-induced anemia and reduction of tissue ascorbate was, however, reduced in rats by ascorbate supplementation, and ascorbic acid synthesis was higher in livers of lead-poisoned rats (Pal et al. 1975). Holmes et al. (1939) provided evidence for reduced severity of lead toxicity in metal workers after ascorbic acid therapy, but this was not the case for workers in a tetraethyl lead plant (Evans et al. 1943). These contradictory findings may be a result of an improvement in overall health of lead-poisoned patients, previously having a poor diet, as well as a lead-chelating action of ascorbic acid similar to that of EDTA (ethylenediaminetetraacetic acid) (Goyer and Cherian 1978). Ascorbic acid alone and in combination has been shown to increase lead loss from tissues of rats (Goyer and Cherian 1978) and metal workers (Papaioannou and Sohler 1978). Therefore, there may not be a direct metabolic interaction between lead and ascorbic acid, but rather a chelating action of excess ascorbate that assists lead excretion. In this experiment, ascorbate did not reduce blood lead of fish probably due to very high lead uptake rates (Hodson et al. 1977) that would "swamp" any excretion.

The appearance of spinal deformities in the lead treated groups and the apparent absence of direct interaction with ascorbic acid suggests that other causative factors produce these symptoms in trout. Kloppel and Post (1975) noted that tryptophan deficiency in rainbow trout led to a transient lordosis and scoliosis, which was initially reversible. They suggested that spinal deformities may have resulted from a lack of serotonin. The hormone melatonin (*N*-acetyl-5-methoxyserotonin), a compound responsible for lightening the color of melanocytes, is also synthesized from tryptophan by way of serotonin (Harper 1973). Thus, it is possible that the etiology of lead toxicity may be related to the metabolism of tryptophan and deficiencies of serotonin and melatonin. Fati (1963) found that tryptophan supplementation (Vitamin B6) of diets of lead-poisoned rats reduced urinary excretion of xanthurenic acid and protoporphyrins. He speculated that lead may interfere with synthesis of nicotinic acid from tryptophan with resultant excretion of xanthurenic acid, an intermediate. Ghelburg et al. (1966) noted that urinary 5-hydroxy indoleacetic acid excretion increased proportionately with lead exposure in children. They interpreted these results as indicating interference with tryptophan metabolism. Obviously, further research is necessary to elucidate the etiology of lead toxicity and its relationship to tryptophan metabolism in rainbow trout.

Acknowledgments

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Feeding Ecology and Vertical Migration of Adult Alewives (*Alosa pseudoharengus*) in Lake Michigan[1]

JOHN JANSSEN[2]

Center for Great Lakes Studies, University of Wisconsin–Milwaukee, Milwaukee, WI 53201, USA

STEPHEN B. BRANDT[3]

Laboratory of Limnology, University of Wisconsin–Madison, Madison, WI 53706, USA

JANSSEN, J., AND S. B. BRANDT. 1980. Feeding ecology and vertical migration of adult alewives (*Alosa pseudoharengus*) in Lake Michigan. Can. J. Fish. Aquat. Sci. 37: 177–184.

Vertical distributions of adult alewife (135–216 mm long) and *Mysis relicta* were measured acoustically on a 24 h basis during July, September, and October 1975 and June 1976 at a station 8 km northeast of Milwaukee, Wisconsin in 50 m of water. Both *Mysis* and adult alewife concentrated on the bottom during day and migrated upwards to the base of the thermocline at night. Extent and timing of vertical migrations of *Mysis* and alewife coincided. During bright moonlight *Mysis* concentrated at depths well below the thermocline and larger adult alewives concentrated within this *Mysis* layer while smaller adult alewives migrated to the base of the thermocline. An examination of stomach contents indicated that vertical migrations were mechanistically linked to feeding behavior. Adult alewives fed extensively on *Mysis* at night. Total lengths of *Mysis* in alewife stomachs were significantly longer than total lengths of *Mysis* captured in vertical plankton tows. *Pontoporeia hoyi* occurred in alewife stomachs in late afternoon (September) and early evening (June, October). Microcrustacean zooplankton were eaten mostly during day (June) or early morning (October). As *Mysis* was the most important food, the vertical migration of alewives is interpreted as an adaptation for feeding on vertically migrating *Mysis*.

Key words: alewives, Lake Michigan, *Mysis*, *Pontoporeia hoyi*, vertical migration, feeding ecology, light

JANSSEN, J., AND S. B. BRANDT. 1980. Feeding ecology and verticla migration of adult alewives (*Alosa pseudoharengus*) in Lake Michigan. Can. J. Fish. Aquat. Sci. 37: 177–184.

Des méthodes acoustiques ont été utilisées pour mesurer la distribution verticale de gaspareaux adultes (135–216 mm de long) et de *Mysis relicta* sur base de 24 h en juillet, septembre et octobre 1975 et en juin 1976 à une station située à 8 km au nord-est de Milwaukee, dans le Wisconsin, à une profondeur d'eau de 50 m. *Mysis* et gaspareaux adultes se concentrent, les uns et les autres, sur le fond durant la journée et remontent à la base de la thermocline la nuit. L'étendue et la chronologie des migrations verticales de *Mysis* et du gaspareau coincident. Par nuits de clair de lune brillant, les *Mysis* se concentrent à des profondeurs bien au-dessous de la thermocline, et les grands gaspareaux adultes se trouvent parmi cette couche de *Mysis*, alors que les gaspareaux adultes plus petits émigrent vers la base de la thermocline. Un examen des contenus stomacaux indique un lien mécanique entre migrations verticales et comportement alimentaire. Les gaspareaux adultes se nourrissent intensivement de *Mysis* la nuit. La longueur totale des *Mysis* présents dans les estomacs de gaspareaux est nettement plus grande que celle des *Mysis* capturés dans des prises de plancton verticales. *Pontoporeia hoyi* est présent dans les estomacs de gaspareaux en fin d'après-midi (septembre) et début de soirée (juin, octobre). Les microcrustacés planctoniques sont mangés surtout de jour (juin) ou tôt le matin (octobre). Comme *Mysis* est la plus importante nourriture des gaspareaux, la migration verticale de ces derniers est interprétée comme adaptation à une alimentation à même des *Mysis* se déplaçant verticalement.

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[1]Both authors made equal contribution; Contribution No. 196, Center for Great Lakes Studies, University of Wisconsin–Milwaukee, Milwaukee, Wisc. 53201, USA.

[2]Present address: Loyola University of Chicago, 6525 North Sheridan Road, Chicago, IL 60626, USA.

[3]Present address: CSIRO, Division of Fisheries and Oceanography, P.O. Box 21, Cronulla, N.S.W. 2230, Australia.

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THE alewife (*Alosa pseudoharengus*) is an exotic fish species in Lake Michigan. Since their first recorded appearance in 1949 (Miller 1957), they spread throughout the lake to become the most abundant species (Brown 1968, 1972; Wells and McLain 1973). The invading alewife undoubtedly affected native fish stocks and the size distribution of zooplankton in Lake Michigan (Smith 1968, 1970; Wells and McLain 1973; Wells 1970). Although mechanisms of these effects are still largely undetermined, we suspect that they may be primarily mediated through the feeding ecology of alewives.

To help define the functional role of these clupeids in the Lake Michigan ecosystem, we examined changes in vertical distribution and food of adult alewives over 24-h periods. Alewives in Lake Michigan eat almost exclusively crustacea ranging in size from small copepods and cladocera to *Pontoporeia hoyi* and *Mysis relicta* (Morsell and Norden 1968; Rasmussen 1973; Webb and McComish 1974; Rhodes and McComish 1975). Other organisms such as fingernail clams, mites, aquatic insects, fish eggs, oligochaetes, leeches, and filamentous algae are also consumed, but only in small quantities. Many potential prey of alewives in Lake Michigan concentrate at certain depths, and their depth distribution changes with time of day and the thermal structure of the water column (Beeton 1960; Wells 1960; Bowers and Grossnickle 1978). The interactions of alewives with these organisms in the pelagic zone have not been described.

In this study we sought to determine if alewives vertically migrated, whether this correlated with vertical distribution of prey, particularly *Mysis*, and whether the migration was related to diel feeding activity.

Materials and Methods

We measured the vertical distributions of fish and *Mysis* acoustically and analyzed the stomach contents of alewives caught in trawls at 4-h intervals or less during 24-h periods on July 22–23, 1975; September 15–16, 1975; October 14–15, 1975; and June 7–8, 1976. Samples were collected at a sampling station 8 km northeast of Milwaukee, Wisconsin at a depth of 50 m from the University of Wisconsin's R/V *Aquarius*. Thermal profiles were measured with a 60 m mechanical bathythermograph and temperatures were read at 1-m intervals to the nearest 0.1°C. In October at dawn and dusk, we measured the surface light intensity with a Whitney light meter (ca. 1960 model) every 2.5 min while pelagic fish distributions were recorded acoustically.

Fish Distribution

Fish samples were collected with a semiballoon otter trawl during 10–30 min bottom tows at speeds of approximately 1.5 m/s (3 knots). The trawl had a 7.6 m headrope, 2 cm bar mesh, and a 0.6 cm mesh liner in the cod end. All fish caught were identified and counted. Twenty or more (unless fewer were available) alewives were preserved for stomach analyses in 10% formalin after cutting the body wall. Catch per unit effort (CPUE) of alewives is expressed as the number caught per 10 min towed on the bottom.

During October, the otter trawl was also fished in midwater to identify pelagic fish targets recorded on the echo sounder. The midwater depth of the trawl was estimated from the length and angle of the cable that had been left out.

For quantitative analyses of midwater fish distributions in October and July, a high resolution Simrad EK-120 echo sounder was used for sound transmission and reception. This echo sounder operates at a frequency of 120 kHz and transmits sound pulses of 0.1 ms duration at a rate of 96 pulses/s. The short pulse length provides a theoretical vertical resolution of 0.075 m. The transducer beam angle for this instrument approximates 11° at −3 db points. We towed the transducer alongside the vessel within a hydrodynamically stable 1.2 m V-fin "fish" at a depth of 1 m.

Acoustic signals were bandshifted to 12 kHz, amplified, and then recorded on one channel of a magnetic tape recorder (Sony, model 277-4) during all trawl tows. The trigger signal, which initiates sound transmission at each acoustic pulse, was amplified and recorded on a second channel of the tape recorder. Later ashore, analog data from the tapes were high pass filtered, rectified, low pass filtered ("envelope detected"), and then input to a 24 K Datacraft (model 6024/5) computer. The high pass filter reduced 60 cycle noise. Signals from a time-mark generator were used to calibrate the filters and rectifier. Data were digitized by the computer and corrected for signal attenuation and spreading loss (Forbes and Nakken 1972; Clay and Medwin 1977). Data were then analyzed by echo-squared integration to provide measures of relative fish abundance for each 1 m depth stratum throughout the water column for each 2 min of transect time. Results from echo-squared integration are directly proportional to absolute fish densities (Thorne 1971). This entire analysis procedure is performed in real time and is fully described in Brandt (1975, 1978) and Peterson et al. (1976).

During September and June, echograms from a 50 kHz Furuno (model F-836) echo sounder were used to monitor vertical distributions of fish. The transducer for this echo sounder was mounted in the hull of the R/V *Aquarius*.

Mysis Distribution

We collected triplicate samples of *Mysis* at night towing a 0.5 m plankton net (#0 mesh = 0.571 mm) vertically from bottom to surface at a constant speed. All zooplankton were preserved in 10% formalin. All *Mysis* were counted and measured for total lengths.

We also measured the vertical distribution of *Mysis* in the water column before starting each trawl tow and during additional periods at dawn and dusk with a Furuno 850-A echo sounder. This 200 kHz echo sounder can record targets as small as *Mysis* and was used successfully for studies of *Mysis* distribution by Teraguchi et al. (1972) and Bowers and Grossnickle (1978). Measurements were taken with the transducer suspended off the side of the ship while the ship drifted. Echograms were used to determine the depth of *Mysis* concentrations. Fish targets are also visible on the echogram but are distinct from those of the *Mysis*. Because we did not correct for differences in sampling volume that resulted from the spherical spreading of sound from the transducer with depth, the targets are disproportionately more numerous near the bottom of the echogram.

Feeding of Alewives

For each preserved alewife, all whole *Mysis, Pontoporeia,* and microcrustacean zooplankters in the cardiac region of the stomach were counted. Percent occurrence (percent of fish containing the item) and number of food items per stomach (median and upper and lower quartiles) were used as qualitative and quantitative indicators of alewife feeding habits. Total length (mm) of each alewife and all *Mysis* from each stomach sample were measured.

Results

Vertical Migration of Alewife

Echosounding in July and October indicated a greater number of fish in the water column at night than during the day. This correlated inversely with a decrease in the number of adult alewives captured in night trawls relative to day trawls (Fig. 1). Significantly more fish were pelagic at night than during the day (Mann-Whitney U-test, $n = 6$ night estimates, 7 day estimates, $P < 0.05$). Echograms for June and September also indicated that substantially more fish were in the water column at night. During the daytime, fish were extremely scarce in the water column on all sampling dates, although some large schools were recorded from the epilimnion in July and September.

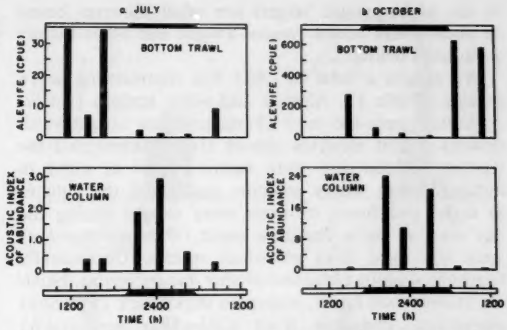

FIG. 1. Alewife catch per unit effort (CPUE) in bottom trawls and acoustic index of fish abundance in the water column (mean echo squared integration) over 24-h periods in July and October 1975 at a station approximately 8 km northeast of Milwaukee, Wisconsin.

While the echo sounder can show fish depth distribution, it cannot indicate species. We believe that most

TABLE 1. Comparison of percent species composition of fishes caught in bottom trawls during day and night for July, September, and October 1975 and June 1976. The sampling station was 8 km northeast of Milwaukee, Wisconsin in Lake Michigan at a water depth of 50 m.

Dates	Total trawl length (min)	Beginning times	Percent of total numbers								Total number of fish
			Alewife		Slimy sculpin	Smelt	Bloater	Fourhorn sculpin	Ninespine stickleback	Lake trout	
			Adult	Juvenile							
July 22–23, 1975	65 Day	1111 1358 1606 0807	18	11	71	0	<1	<1	0	0	717
	50 Night	2108 0013 0410	1	<1	99	0	0	0	0	0	1686
Sept. 15–16, 1975	30 Day	1458 0658 1108	39	0	37	21	2	0	1	0	346
	30 Night	1928 0053	<1	0	99	<1	0	0	0	0	175
Oct. 14–15, 1975	31 Day	1513 0708 1105	94	0	<1	5	<1	0	<1	<1	1694
	40 Night	1909 2308 0307	24	0	73	3	0	0	<1	0	418
June 7–8, 1976	40 Day	1525 1936 0532 1119	35	53	7	5	<1	0	0	0	1742
	40 Night	2049 0211	14	0	85	<1	0	<1	0	0	857

of the night pelagic targets are adult alewives based on abundances of fish species caught and other factors as outlined below.

We caught a total of 7635 fish representing seven species (Table 1). Alewife and slimy sculpin (*Cottus cognatus*) were the most abundant fishes caught in all seasons. Adult alewives almost always dominated the daytime catches but were rarely caught at night in bottom trawls. Slimy sculpins dominated the catches at night and fewer of them were caught during the day than at night. Rainbow smelt (*Osmerus mordax*) were the third most abundant species. Occasionally fourhorn sculpin (*Myoxocephalus quadricornis*), bloaters (*Coregonus hoyi*), ninespine stickleback (*Pungitius pungitius*), and lake trout (*Salvelinus namaycush*) were also caught. Most alewives in the catch were age II or older, ranging in length from 135 to 216 mm for all dates and times combined. The absence of juvenile alewives in bottom trawls is consistent with reports that the juveniles are predominantly pelagic (Wells 1968a; Brown 1972; Brandt 1978).

We concluded that the pelagic fish in the water column at night were mainly adult alewives because (1) the adults were the most abundant fish on the bottom during the day (Table 1), (2) the reduction of adult alewives on the bottom at night corresponded with an increase of fish in the water column (Fig. 1), (3) the only fish we captured in midwater trawling at night were adult alewives, and (4) the only other abundant species, the slimy sculpin, was most abundant in bottom trawl catches at night (Table 1), and having no swim bladder, is less likely than the alewife to be in midwater or to register on an echo sounder.

Vertical migration of adult alewives is further corroborated by the progressive shifts in vertical distribution of fish at dawn and dusk, coinciding with changes in surface light intensity. For example, during October, fish began to migrate toward the bottom at surface light intensities of less than 10 lx (Fig. 2), and the migrations were complete at surface light intensities of approximately 400 lx. The rate of downward migration was estimated to be 0.85 m/min. Similar migration patterns were observed during other months.

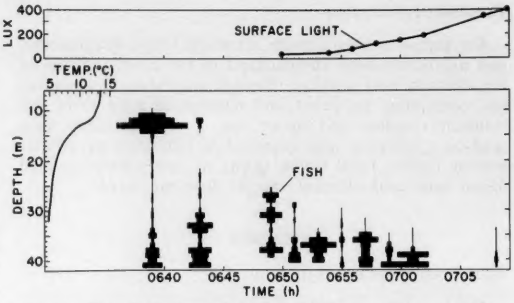

FIG. 2. Relative abundance of fish throughout the water column and corresponding surface light intensities during dawn on October 15, 1975. Horizontal bars represent relative fish abundance as measured by echo squared integration. Fish are believed to be predominantly adult alewife.

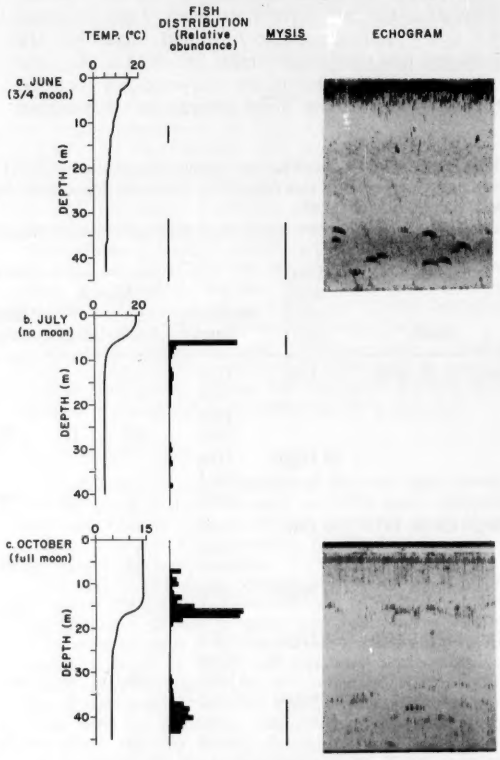

FIG. 3. Nocturnal vertical distributions of water temperature, relative fish abundance from echo squared integration, and *Mysis* from echograms from a 200 kHz Furuno 850-A echo sounder in Lake Michigan on (a) June 8, 1976 (03:30), (b) July 22, 1975 (21:22), and (c) October 14, 1975 (23:08).

VERTICAL DISTRIBUTION OF MYSIS AND FISH

The night vertical plankton tows yielded *Mysis* abundance estimates of 522/m^2 in July, 190/m^2 in September, 230/m^2 in October, and 165/m^2 in June.

The extent and timing of vertical migrations of fish were closely linked to thermal structure and *Mysis* distribution. Regardless of date or habitat conditions, the night time fish targets were always concentrated in the *Mysis* layer (Fig. 3) as determined with the 200 kHz Furuno 850-A echo sounder. On dark nights (i.e. new moon) with a thermocline present (Fig. 3b), both fish and *Mysis* were at the base of the thermocline. On moonlit nights (Fig. 3a, b) *Mysis* did not rise as high in the water column and all or a portion of the

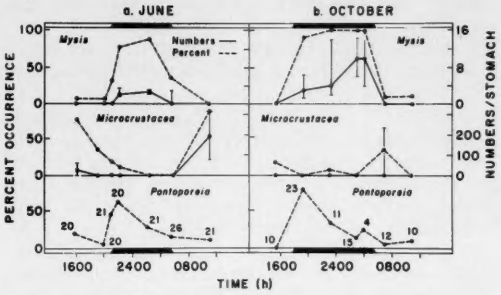

FIG. 4. Numbers per stomach and percent occurrence of *Mysis*, *Pontoporeia*, and microcrustacean zooplankton in alewife stomachs for (a) June 1976 and (b) October 1975 over 24-h periods. Alewives were collected with a bottom trawl 8 km northeast of Milwaukee in Lake Michigan in 50 m of water. Number of stomachs examined are indicated in the bottom panel.

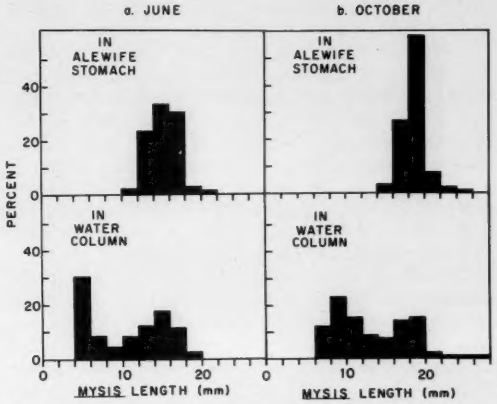

FIG. 5. Length frequency (percent) distributions of *Mysis* from stomachs of adult alewife and from the water column for (a) June 1976 and (b) October 1975 at a station 8 km northeast of Milwaukee in Lake Michigan in 50 m of water.

fish population remained with the *Mysis* deep in the water column. In October, however (Fig. 3c), the smaller adult alewives apparently migrated past the *Mysis* layer up to the base of the thermocline. Mean length of alewives caught near the bottom in the *Mysis* layer at night was significantly longer than that of alewives caught during the day when all fish were near the bottom ($P < 0.05$ median test, $n = 48$ fish at night, 42 during day). This indicates that smaller alewives migrated higher in the water column than did larger alewives.

Both fish and *Mysis* migrated to the bottom at dawn, and the vertical migration of the fish coincided with that of the *Mysis*. The downward rate of migration for *Mysis* in September, for example, was 0.7 m/min, similar to that of the fish.

Feeding of Alewives

Adult alewives fed intensively on *Mysis* at night (Fig. 4). Both numbers of *Mysis* per stomach (Kruskal–Wallis analysis of variance by ranks and Dunn's (1964) multiple comparison tests, $P < 0.1$) and percent occurrence of *Mysis* in alewife stomachs (χ^2 and Fisher exact test) were significantly higher at night than during day in June, September, and October. Day–night stomach comparisons were not made for July because too few alewives were caught in trawls at night.

Mysis were present in the stomachs of an average of 13% of the alewives sampled during the day and 85% at night in June, and 9% sampled during the day and 96% at night in October. Median numbers per stomach were near zero during the day but increased at night to 2 in June and 10 in October. In September, many alewives (77%) contained *Mysis* during the day but the number per stomach was higher at night (maximum 8/stomach) and in early morning (7/stomach) than later in the day (one to two/stomach). Although few alewives were actually caught in midwater, four taken during a 37 min midwater-trawl tow at night in October contained 4–19 *Mysis*. These values are similar to those from alewives caught in bottom trawls at night.

The number of *Mysis* per stomach was extremely variable within trawl samples as indicated by the breadth of the quartiles (Fig. 4). Variance to mean ratios for June, September, and October night samples ($n = 9$) ranged from 1.33 to 6.47 (median = 3.89). In eight samples the variance was significantly greater than the mean ($P < 0.05$, χ^2 test on index of dispersion; Elliot 1971). The possible importance of this observation is considered in the discussion.

Body lengths of *Mysis* in alewife stomachs were longer than body lengths of *Mysis* in the water column in June and October (Fig. 5) and also in September (median test, $n = 272$-341 *Mysis* in sample, 115–269 in stomachs, $P < 0.05$).

Pontoporeia were less common prey of alewives than were *Mysis* (Fig. 4). The median number of *Pontoporeia* per stomach was always zero and total numbers ranged from 0 to 9. The highest percentage of *Pontoporeia* occurred in the stomachs sampled during the afternoon in September (60%) as compared to early evening in October (78%) and June (65%). Few alewives contained *Pontoporeia* during the day.

Microcrustacean zooplankton found in the alewife stomachs were mainly cyclopoid and calanoid copepods and *Daphnia* and *Bosmina* sp. These organisms were relatively uncommon in the stomachs of the adult alewives but did appear in moderate numbers in those of fish from certain trawls in June and October (Fig. 4). The greatest number per stomach was generally observed during the day.

The high numbers of microcrustaceans in stomachs

of fish caught in midwater in October suggests that alewives in the thermocline were feeding on zooplankton at night. In fact, the four fish caught near the thermocline in midwater at night all contained zooplankton (median 60.5 zooplankters per stomach, range 23–75) and had significantly higher numbers of zooplankters than fish caught at all other times except early morning (07:08). Midwater fish also contained numerous *Mysis*. Those alewives caught in bottom trawls in the early morning that contained zooplankton had probably fed in the thermocline during the night and then migrated to the bottom at dawn.

The relative importance of *Mysis* in the alewife diet can be estimated using the dry weights of prey measured by Morsell and Norden (1968). Their results state that dry weight on 1 *Mysis* is roughly equivalent to that of 1000 zooplankters. The maximum number of zooplankters (580) taken from an alewife's stomach in our study approximated 0.6 *Mysis* in weight.

Discussion

Adult alewives migrate vertically in Lake Michigan. They concentrated near bottom during the day and in midwater at night. The upper limit to vertical migration was closely linked to the vertical distribution of *Mysis* and the position of the thermocline. When *Mysis* migrated to the base of the thermocline, as they do if moonlight is not great (Beeton 1960; Bowers and Grossnickle 1978), alewives also migrated to the base of the thermocline and remained there throughout the night. If, on the other hand, the upward migration of *Mysis* was limited to a region well below the thermocline, as it was on a moonlight night in October of our study, longer adult alewives still concentrated in the *Mysis* layer. Shorter adults, however, apparently passed through the *Mysis* layer and concentrated at the base of the thermocline where *Mysis* are scarce, presumably to feed on microcrustacea. Differences in vertical distribution of different size-classes of fish have been documented in marine systems (Clarke 1973, 1974; Clarke and Wagner 1976), but the adaptive significance is unclear.

We suggest that the extent of the vertical migration by alewives is mechanistically linked to their feeding behavior. Similar vertical distributions and migration rates of both adult alewives and *Mysis* suggest that alewives actually follow *Mysis*. It is unlikely, however, that both alewives and *Mysis* have identical thermal and light preferences and are merely responding similarly to abiotic physical stimuli. During all months except October pelagic alewives were concentrated in the *Mysis* layer. The bimodal distribution of fish during October coincided with the bimodal distribution of their prey, that is, large adult alewives were in the *Mysis* layer whereas smaller adults concentrated with the microcrustaceans at the base of the thermocline (Wells 1960; Lane 1975). It is difficult to explain on the basis of temperature and light characteristics alone, why alewives should have two optima on one data and only one on another.

Our Lake Michigan data depict *Mysis* as the major constituent in the diet of adult alewives at the offshore station in 50 m of water. Alewives fed on *Mysis* almost exclusively at night. Previous studies of food habits of hypolimnetic alewives (Morsell and Norden 1968; Rasmussen 1973) suggested that *Pontoporeia* were the most important prey, but those fish were sampled mainly during daylight hours. Apparently *Mysis* are more vulnerable to alewife predation in the water column that near bottom. Alewives in the laboratory have difficulty feeding from the bottom and *Mysis* visually avoid them (Janssen 1978a). Thus, although both *Mysis* and alewives are most concentrated when on the bottom during the day, the *Mysis* are apparently unavailable then as prey.

Vision may well be the mode by which alewives detect *Mysis* at night. The selection of large *Mysis* by alewives is consistent with the idea that large *Mysis* should be more visible and thus more vulnerable than small *Mysis* to alewife predation. *Mysis* in Lake Michigan prefer light intensities of about 0.1 lx when migrating (McNaught and Hasler 1966), but they were often found at depths with less light. Light intensities of 0.1 lx are well within the visual ability of clupeid. The Atlantic herring (*Clupea harengus harengus*) has a feeding threshold near 0.01 lx (Blaxter 1966). At such low light intensities, the Atlantic herring silhouettes its prey against downwelling light and approaches the prey from beneath. The alewife also approaches individual prey from beneath (Janssen 1978a), and its eye seems to be better adapted than the herring's eye for silhouetting prey, because the concentration of receptors in the ventral part of the retina is higher than that observed in the Atlantic herring (Ali and Anctil 1976). Alewives are also capable of feeding nonselectively in the dark, presumably by filtering (Janssen 1978b). But if alewives were filter feeding at night we would expect them also to pick up microcrustaceans. Indeed, the four alewives captured at night near the thermocline in October did contain substantial numbers of microcrustaceans. However, zooplankters were uncommon in alewife stomachs from samples taken on bottom at night. Perhaps filter feeding is only rewarding in high concentrations of zooplankton such as occur in the thermocline. Age-II and older alewives fed on zooplankton mainly at night during July and August in Echo Lake, Maine (Gately 1978).

Differences in the vertical distribution of large and small *Mysis* may contribute to the apparent "size selective" feeding by alewife at night. Smaller *Mysis* are often higher in the water column than large *Mysis* (Beeton 1960). If alewives fed primarily in the lower part of the *Mysis* layer, stomach samples would be biased toward larger prey.

Numbers of *Mysis* in fish stomachs varied greatly. If alewives were random sampling devices, a large variance/mean ratio in stomach contents would imply

that *Mysis* were distributed in patches. Clearly the *Mysis* are patchily distributed in that they are layered vertically. Night vertical hauls of *Mysis* are reported by N. E. Grossnickle (Harbor Branch Institution, Fort Pierce, FL 33450, USA, personal communication) indicate that they are also distributed horizontally in patches. For 12 out of 15 series of samples collected by Grossnickle at a 50 m station from July 1975 to June 1976, the variance was significantly greater than the mean ($P < 0.05$, χ^2 test on the index of dispersion; Elliot 1971).

As noted earlier, *Pontoporeia* were less important than *Mysis* as alewife prey. *Pontoporeia* are usually buried in the sediments (Wells 1968b) and thus unavailable to alewives. Occasionally, and particularly at night, *Pontoporeia* make short sojourns in the water column (Marzolf 1965), however, and become vulnerable to alewife predation. Our sampling indicated that adult alewives feed on *Pontoporeia* primarily during late afternoon and early evening, apparently during the initial stages of their migration off the bottom. But, alewives are usually above most of the *Pontoporeia* later during the night.

Mysis and *Pontoporeia* are important prey for bloaters (Wells and Beeton 1963), slimy sculpin and deepwater sculpin (Kraft 1977). Foltz and Norden (1977) found that smelt fed largely on *Mysis*. Similarities among the diets of deepwater fishes in Lake Michigan suggest that competition for food may be important; however, we do not actually know if prey are ever in short supply for any of these species.

Spatially, the thermocline represents only a small percentage of the lake, yet recent studies have depicted this zone as a site of intensive interactions between nutrient, phytoplankton, and microcrustacean zooplankters (Mortonson 1977; Brooks and Torke 1977; Wells 1960; Lane 1975). These interactions are obviously highly significant to the productivity of the pelagic waters of Lake Michigan. Bowers and Grossnickle (1978) and Grossnickle (1978) have since added *Mysis* to this dynamic region in Lake Michigan with the observation that *Mysis* migrate to the base of the thermocline at night, if moonlight is not great, and feed on concentrations of diatoms and microcrustaceans. Our data on alewives add one higher trophic level to this complex system.

Acknowledgments

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Factors Controlling Oxygen Depletion in Ice-Covered Lakes

JACK A. MATHIAS AND JAN BARICA

Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man. R3T 2N6

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Winter oxygen depletion rates from four sets of Canadian lakes (prairie, southeastern Ontario, Arctic, and Experimental Lakes Area) differing in morphometry and trophic state, were analyzed. An inverse relationship was found between oxygen depletion rate and mean depth. The effect of lake trophic status on oxygen depletion rate was demonstrable after the influence of basin morphometry was removed by regression of oxygen depletion rate against the sediment area: lake volume ratio. The sediments of eutrophic lakes consumed oxygen about 3 times faster (0.23 $g \cdot m^{-2} \cdot d^{-1}$) than those of oligotrophic lakes (0.08 $g \cdot m^{-2} \cdot d^{-1}$), but water column respiration was about the same (0.01 $g \cdot m^{-3} \cdot d^{-1}$) for both groups of lakes. Data from prairie lakes showed that the winter oxygen consumption was limited by oxygen supply below an average whole-lake oxygen concentration of 3.8 $mg \cdot L^{-1}$. The rate of eddy diffusion near the sediments in ice-covered prairie lakes was $3.72 \pm 1.41 \times 10^{-3}$ $cm^2 \cdot s^{-1}$. Implications for lake management during the winter are discussed.

Key words: oxygen, depletion, respiration, lakes, ice-covered, winter, sediments, model, consumption

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Les taux d'épuisement d'oxygène en hiver dans quatre groupes de lacs canadiens (prairie, sud-est de l'Ontario, Arctique et région des lacs expérimentaux), de morphométrie et de conditions trophiques différentes, ont été analysés. On a trouvé une relation inverse entre taux d'épuisement d'oxygène et profondeur moyenne. L'effet de la condition trophique du lac sur le taux d'épuisement d'oxygène a pu être démontré après avoir éliminé l'influence de la morphométrie du bassin par régression du taux d'épuisement d'oxygène contre le rapport superficie des sédiments: volume du lac. Les sédiments de lacs eutrophes consomment de l'oxygène environ 3 fois plus rapidement (0,23 $g \cdot m^{-2} \cdot d^{-1}$) que ceux des lacs oligotrophes (0,03 $g \cdot m^{-2} \cdot d^{-1}$), mais la respiration dans la colonne d'eau est à peu près la même (0,01 $g \cdot m^{-3} \cdot d^{-1}$) pour les deux groupes de lacs.

Les données sur les lacs de prairie démontrent que la consommation hivernale d'oxygène est limitée par les apports d'oxygène au-dessous d'une concentration moyenne, pour tout le lac, de 3,8 $mg \cdot L^{-1}$. Le taux de diffusion tourbillonnaire près des sédiments dans les lacs de prairie recouverts de glace est de $3,72 \pm 1,41 \times 10^{-3}$ $cm^2 \cdot s^{-1}$. Nous analysons les implications quant à la gestion des lacs en hiver.

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THE rate of oxygen depletion in ice-covered lakes is of practical interest because of the fish "winterkill" phenomenon, and of theoretical interest because it relates to lake metabolism. Recent summaries of winter oxygen depletion rates (Schindler 1971a; Welch 1974; Welch et al. 1976; Barica and Mathias 1979) have attempted to demonstrate their dependence upon either morphometric variables or indices related to lake trophic status. These attempts, although partially successful (Table 1), have led to a number of conflicting interpretations as to what the controlling variables might be. In this paper we attempt to clarify the problem by demonstrating the importance of both lake morphometry and trophic state.

Welch et al. (1976) found that the winter respiration rates of 16 Ontario lakes, calculated per unit area of lake surface, were correlated strongly with summer chlorophyll *a* per square metre and spring total phosphorus per square metre. Among morphometric parameters, however, an even stronger correlation was found with mean depth. When morphometric and nonmorphometric parameters were combined, the contribution of chlorophyll *a* and phosphorus became negligible (Table 1). Other variables such as Secchi depth and

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TABLE 1. The usefulness of various morphometric and trophic indices in estimating the rate of winter oxygen depletion ($g \cdot m^{-2} \cdot d^{-1}$) in different lake sets. (+) indicates significant correlation at the 5% level; (−) indicates no correlation; 0 indicates that correlation was not attempted.

Index	Lakes			
	Ontario	ELA	Arctic	Prairie
$\frac{A_d + A_o}{V}$	0	(+)	(−)	(−)
$\bar{z}$	(+)	(−)	(−)	(+)
Chl *a* and P	(+)	(−)	0	(−)
Conductivity	0	0	(+)	(−)

the N:P ratio, were poorly correlated with winter oxygen depletion rates.

Schindler (1971b) correlated winter oxygen depletion rates in 11 lakes in the Experimental Lakes Area (ELA) on the Canadian Shield with the ratio $A_d + A_o/V$, where A_d is the area of the drainage basin, A_o the area of the lake, and V the lake volume. This ratio was taken to be an index of the rate of supply of nutrients and allochthonous carbon to the lakes, adjusted for dilution by their volume. Probably because vegetation cover and land use differ among drainage basins in the Arctic lakes and prairie kettle lakes, the $A_d + A_o/V$ ratio does not apply to them (Welch 1974; Barica and Mathias 1979). Conversely, oxygen depletion rates of ELA lakes were poorly correlated with mean depth ($r = 0.43$, calculated from Schindler 1971a), and with maximum depth, summer phosphorus per square metre, and chlorophyll per square metre, ($r = 0.37$, $r = 0.46$, and $r = 0.04$, respectively; Welch et al. 1976).

In the 10 prairie pothole lakes studied by Barica and Mathias (1979), winter oxygen depletion rate per square metre was strongly correlated with mean depth ($r = 0.92$) but not with total dissolved solids, chlorophyll, or nutrient status.

The significance of these various parameters for the four sets of data is briefly summarized in Table 1. Although winter respiration in each of the four lake sets fits either a morphometric model or a nutrient status model, neither explains all four lake sets well. We reanalyzed the data to show that after the influence of basin morphometry is separated out by regression, the influence of lake trophic status is apparent.

Materials and Methods

Winter oxygen depletion rates ($g \cdot m^{-2} \cdot g^{-1}$) for prairie lakes were taken from Barica and Mathias (1979). They were reanalyzed on a cubic meter basis as follows. The volume of each 1 m layer of the lake was multiplied by its mean dissolved oxygen concentration ($g \cdot m^{-3}$), and the products were summed over all layers to yield the total mass of oxygen in the lake. This mass was divided by total lake volume at the time of freeze-up, to express an oxygen concentration ($g \cdot m^{-3}$) per lake, and this concentration was regressed against time (r always greater than 0.95) to give the rate of oxygen depletion per day. Here, we did not correct lake volumes throughout the winter for ice thickness as was done in Barica and Mathias (1979), because we required a measure of oxygen which would remain constant in the hypothetical case of zero oxygen consumption. Mass of oxygen per uncorrected volume meets this criterion, whereas mass per volume corrected for ice thickness (actual mean concentration for the whole lake) would tend to increase as lake volume decreased. Values of sediment area:lake volume were calculated using mean lake area and mean lake volume averaged over the period of oxygen consumption measurement. Both volume and area varied as ice formation proceeded and were corrected accordingly.

Respiration rates for Arctic lakes were taken from Welch (1974) for those lakes where mean depths were given. We excluded Keyhole Lake, Victoria Island, because its respiration rate was based on an assumed oxygen concentration. We converted Welch's respiration rates per square metre of total surface area of the lake to units of mass of oxygen per cubic metre (uncorrected whole-lake volume) per day, and corrected the sediment area:lake volume parameter for an average ice thickness of 1.1 m (see Welch's fig. 2).

Oxygen depletion rates for ELA lakes and one Minnesota lake were taken from Schindler (1971a). In addition we have included more recent measurements from ELA lakes (D. W. Schindler unpublished data). The sediment area:lake volume ratios were corrected for actual measured ice thickness in the recent data, and for an estimated mean ice thickness of 40 cm in the older data. Morphometric data were taken from Brunskill and Schindler (1971).

Data for Ontario lakes were taken from Welch et al. (1976) and converted to rate units of mass of oxygen per cubic metre per day. The mean depth of only the shallowest lake (Cranberry) was corrected for ice thickness.

Table 2 lists the area of each lake, the year it was

TABLE 2. Areas and mean summer chlorophyll *a* concentrations in the euphotic zone of lakes. (Prairie lakes, J. Barica [unpublished data]; Arctic lakes, Kalff and Welch [1974]; Ontario lakes, Dillon and Rigler [1974]; ELA lakes, [a]epilimnion values, Fee [1979], [b]1968 data, Armstrong and Schindler [1971], [c]average values for 1969–72, Reid et al. [1975].)

Index no.	Lake	Year	Area (ha)	Chl. *a* ($mg \cdot m^{-3}$)
Prairie lakes				
1	885	74	2.28	26.5
2		75		38.5
3		76		102.4
4	255	74	3.44	26.7
5		75		9.3
6		76		5.0
7	318	74	18.6	13.6
8		75		55.0
9		76		11.7
10	587	74	6.88	28.8
11		75		22.4
12		76		29.4
13	721	74	7.09	13.3
14		75		6.6
15		76		6.1

TABLE 2 (*Concluded*)

Index no.	Lake	Year	Area (ha)	Chl. *a* (mg·m^{-3})
16	675	74	9.12	17.3
17		75		12.4
18		76		18.1
19	882	75	5.97	30.7
20		76		51.5
21	200	76	9.87	16.5
22	019	75	28.70	13.6
23	100	75	27.30	19.2
Arctic lakes				
24	Char	69–71	52.6	0.1–0.7
25	Water	61–62	—	
26	Meretta	69–71	20.0	3–5
27	Eleanor	71–72	—	
28	Resolute	71	119.2	
ELA lakes				
29	122	69	12.20	[b]1–5
30	132	68	7.17	[b]1–3
31		69		
32	227	68	5.00	[b]2–5
33		69		[c]92.5
34		70		[c]92.5
35		72		[c]92.5
36		74		[a]34.3
37		76		[a]23.0
38	230	70	1.67	[c]5.4
39	239	68	56.10	[b]1–2
40		69		[c]3.0
41	240	68	44.10	[b]2
42		69		[c]3.2
43	261	70	5.57	[c]4.8 [b]1–2
44		73		[a]4.3
45		74		[a]6.5
46	265	70	13.10	[c]4.0
47	303	69	9.93	[c]4.8
48		70		[c]4.8
49		71		[c]4.8
50		74		—
51	304	69	3.67	[b]1.0 [c]11.3
52	305	68	52.00	[b]1.0
53		69		[c]2.3
54	Severson	64	—	30–300
Ontario lakes				
55	Beech	72	136	1.03
56	Bob	72	227	1.23
57	Boshkung	72	708	1.09
58	Cameron	72	1272	1.60
59	Cranberry	72	71	1.00
60	Eagle	72	233	1.75
61	Four Mile	72	773	1.43
62	Green	72	122	1.52
63	Haliburton	72	285	0.96
64	Hall's	72	532	0.75
65	L.Boshkung	72	127	1.76
66	Maple	72	297	1.08
67	Moose	72	285	1.07
68	Oblong	72	91	2.09
69	Pine	72	114	1.15
70	12 Mile	72	349	1.33

sampled, the mean summer chlorophyll *a* concentration and an index number which identifies it in Fig. 2. Values for oxygen depletion rates per square metre and standard mean depths can be found in the papers cited.

In all of the prairie lakes, dissolved oxygen was measured at 1-m depth intervals throughout the period of ice cover (Barica and Mathias 1979). The deepest measurement in each lake, however, was always taken at approximately 30 cm above the sediment surface, and this value was used in estimating the dissolved oxygen gradient near the sediment surface.

Results

Volume-based oxygen consumption in ice-covered lakes shows a strong inverse correlation with mean depth (Fig. 1). The relationship is of the form $Y = 1/X$, as shown by the two curved lines. These lines, representing area-based oxygen consumption rates of 0.4 g·m^{-2}·d^{-1} and 0.08 g·m^{-2}·d^{-1}, encompass almost the entire range of data points. Mean depth, however, has limited usefulness for expressing winter respiration rates because it offers no means for distinguishing between productive and less productive lakes. As seen in Fig. 1, their rates can vary by a factor of 5 when the mean depth is small. Furthermore, demonstrations of a relationship between area-based respiration rates and mean depth (Table 1) are strictly empirical. There has been no logic advanced which would provide a theoretical basis for the observations.

We have assumed that the sediments of a lake are the primary site of biological and chemical oxidation in winter, and that water mixing under ice cover is sufficient to maintain an adequate oxygen concentration at the sediment surface. Under these assumptions, the rate of oxygen depletion of a unit volume of water in an ice-covered lake should be directly proportional to the average surface area of sediment in contact with that unit volume. This relationship is clearly demonstrated in Fig. 2 in which eutrophic prairie lakes are differentiated from oligotrophic lakes situated on the Canadian Shield and in the Arctic, most of which lie in uninhabited drainage basins.

The lakes shown in Fig. 2 have been classed as oligotrophic or eutrophic largely on the basis of mean summer chlorophyll *a* concentrations in the euphotic zone (Table 2). Where these measurements were lacking or ambiguous, other factors such as average phytoplankton biomass, and annual primary productivity were considered.

The prairie lakes are clearly eutrophic with mean summer chlorophyll *a* concentrations ranging from 13.6 to 102 mg·L^{-1} (J. Barica unpublished data). Although some lakes had mean chlorophyll *a* concentrations as low as 5 and 6 mg·L^{-1} in some years (lakes 255 and 721), maximum values were always greater than 10 mg·L^{-1} (Barica and Mathias 1979; Barica et al. 1978). Ontario lakes can be considered uniformly oligotrophic.

Among the ELA lakes, two have been made eutrophic by the experimental addition of nutrients since 1969

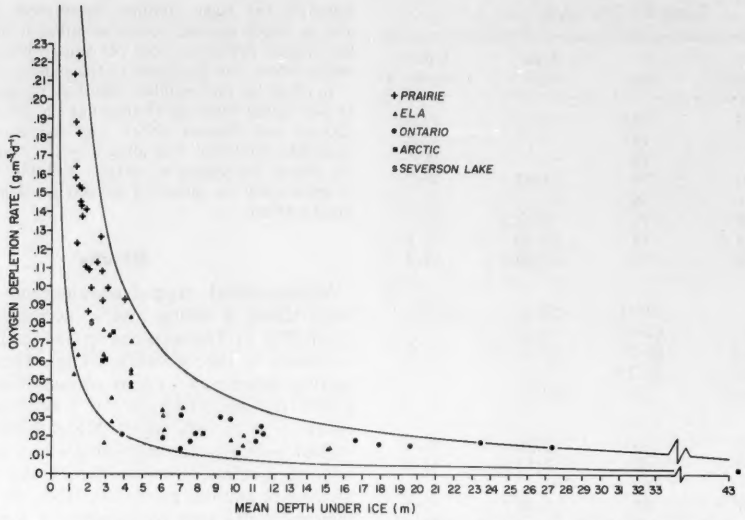

FIG. 1. Winter rate of oxygen depletion as a function of mean lake depth corrected for ice thickness. For comparison, lines indicate the loci of lakes whose area-based respiration rates are constant at 0.40 g O_2 $m^{-2} \cdot d^{-1}$ (upper line) and 0.08 g O_2 $m^{-2} \cdot d^{-1}$ (lower line).

(lake 227) and since 1973 (lake 261). Postfertilization data for these lakes (index no. 33, 34, 35, 36, 37, 44, and 45) have been grouped with the eutrophic lakes. ELA lake 304 (index no. 51) which is unpolluted, is nevertheless classed as eutrophic based on mean summer chlorophyll *a* concentrations, phytoplankton cell biomass, and nutrient dynamics. Although the mean summer chlorophyll in 1968 was only 1.0 mg·m^{-3} (Armstrong and Schindler 1971), it averaged 11.3 mg·m^{-3} from 1969 to 1972 (Reid et al. 1975). This latter value would class lake 304 as mesotrophic to eutrophic according to Sakamoto (1966). Similarly, a phytoplankton biomass of over 10 $cm^3 \cdot m^{-3}$ in July and August 1969 (Schindler and Holmgren 1971) would class lake 304 as eutrophic according to Vollenweider (1968). Armstrong and Schindler (1971) suggest that there is considerable feedback of nutrients from the sediments of this lake. In summer of 1969, the estimated P input from sediments in lake 304 exceeded that from runoff and precipitation by a factor of 7, and exceeded net retention of P (inputs − outputs) by a factor of 21. Thus it is possible that as a result of its shallow depth and resulting sediment nutrient return, lake 304 should be considered eutrophic despite low ambient nutrient concentrations and runoff inputs which would otherwise classify it as oligotrophic. Severson Lake (index no. 54), reported by Schindler and Comita (1972), is classed as eutrophic. In 1955 the mean summer chlorophyll *a* concentration was 300 mg·m^{-3}. In 1964–65 it was only ca. 30 mg·m^{-3}, but gross primary production was similarly high during both years at greater than 5000 kcal·$m^{-2} \cdot yr^{-1}$ (ca. 20 900 kJ·$m^{-2} \cdot yr^{-1}$).

Among the Arctic lakes, Char (index no. 24) is classed as ultraoligotrophic based on maximum or average chlorophyll *a* concentrations and primary production (Kalff and Welch 1974). Although no biomass or production data are available for Eleanor and Resolute lakes (index no. 27 and 28), we assumed them to be oligotrophic also since they lie on the same bedrock as Char Lake, and neither has been exposed to substantial human interference (Welch 1974). Meretta Lake (index no. 26) on the other hand, has been made eutrophic by addition of sewage nutrients, and its metabolic response has been documented by Schindler et al. (1974). Water Lake was classed as eutrophic on the basis of Welch's (1974) description.

The slopes of the lines in Fig. 2 are estimates of the sediment oxygen consumption rate per square metre of the two groups of lakes. Both slopes are statistically significant (differing from a value of 0.0; $P < 0.01$), with correlation coefficients of 0.85 and 0.87 for the eutrophic and oligotrophic lakes, respectively. The two slopes shown in Fig. 2 are also significantly different from each other ($P < 0.01$). Sediments of the oligotrophic lakes consume oxygen at a mean rate of 0.075 g·$m^{-2} \cdot d^{-1}$, about 3 times less than the eutrophic lakes (0.23 g·$m^{-2} \cdot d^{-1}$).

To examine the relation between oxygen depletion rate and oxygen concentration, we replotted data from prairie lakes whose oxygen depletion curves (Barica and Mathias 1979) became curvilinear at oxygen concentrations below about 3.0 mg·L^{-1}. Figure 3 shows

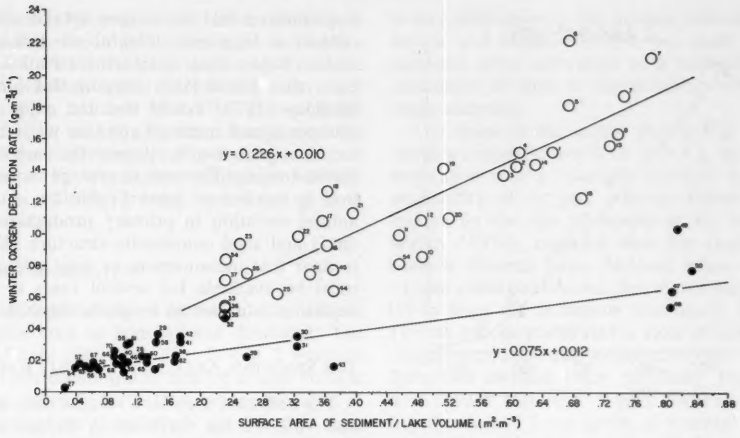

FIG. 2. Relation between winter oxygen depletion rate and the sediment area:lake volume ratio. Upper line describes eutrophic lakes. Lower line describes oligotrophic lakes. Index numbers refer to Table 2. Regression equation for each line is indicated.

rates of oxygen depletion over short periods of time (1–3 wk) plotted against the oxygen concentration of water 30 cm above the sediment at the beginning of each rate measurement. All oxygen depletion rates are expressed per square metre of unfrozen sediment surface.

Figure 4a shows an example of the curvilinear nature of the oxygen depletion curve at oxygen concentrations below about 3 $g \cdot m^{-2}$ (corresponding in this lake to about 2 $mg \cdot L^{-1}$), and Fig. 4b shows the concomitant vertical pattern of oxygen depletion.

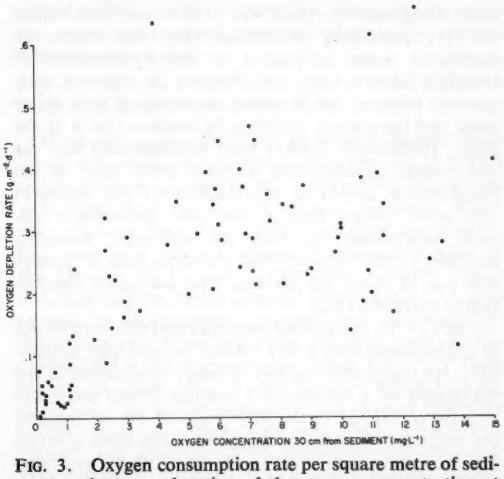

FIG. 3. Oxygen consumption rate per square metre of sediment surface as a function of the oxygen concentration at a point 30 cm above the sediment. Data are for prairie lakes only.

Discussion

The rate of oxygen depletion in ice-covered lakes is probably mediated largely by the bacterial population inhabiting the oxidized microzone of the sediments and most likely depends on basin morphometry, the availability of organic material and oxygen, and the degree of water mixing which brings oxygen into contact with the sediments. These factors are discussed below.

MEAN DEPTH

Classical inverse relationships between mean depth and various measures of lake productivity have been demonstrated by numerous studies. The relationship holds for chlorophyll (Sakamoto 1966), the biomass of zooplankton and benthic fauna (Rawson 1955), and fish yields (Rawson 1952; Larkin 1964). Lake metabolism during the winter apparently follows the same pattern (Fig. 1). Much of these patterns can probably be explained by the fact that primary production in larger lakes takes place primarily in the mixed layer. Since the depth of the mixed layer is relatively independent of lake size (over the range of lake sizes dealt with here), its volume is primarily a function of lake area. This tends to decrease exponentially, relative to total lake volume, as the size and mean depth of lakes increase. Similarly, the area of productive interfaces such as the sediment–water interface and the littoral zone also decrease exponentially relative to lake volume, with increased lake size. The simple inverse relationship found in the oxygen depletion rate data (Fig. 1) suggests the importance of sediment area in relation to water volume, since area/volume is the inverse of mean depth.

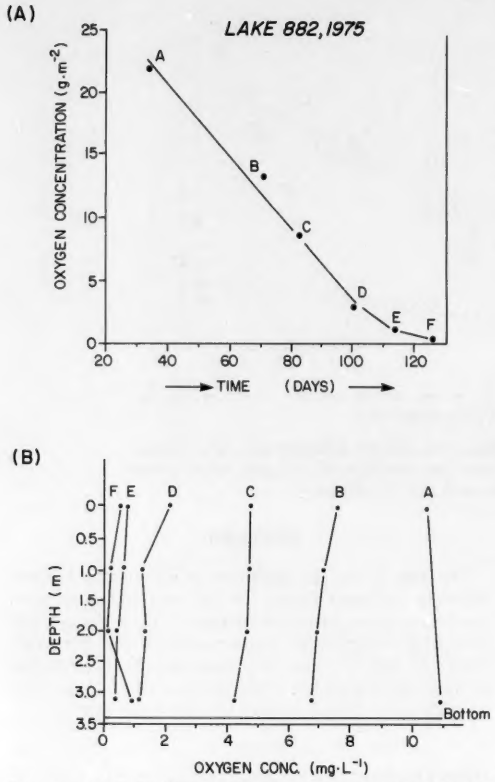

FIG. 4. A) Lake 882, 1975. Mean oxygen concentration as a function of time, from formation of ice cover until point of lowest concentration; B) Winter oxygen profiles for lake 882, 1975. Letters refer each profile to the average oxygen concentration shown above in (A).

LAKE TROPHIC STATE

Figure 2 suggests that more productive lakes exhibit a higher rate of sediment oxygen demand than less productive lakes. It has long been recognized that lake productivity is related to the rate of formation of hypolimnetic oxygen deficits in stratified lakes (Hutchinson 1938, 1957), and this has been used to index the process of lake eutrophication (Bazin and Saunders 1971; Carr 1962). Hargrave (1973) has shown that annual sediment respiration, estimated from oxygen uptake in cores taken from various freshwater and marine ecosystems, was positively related to annual primary production. This relationship has recently been confirmed for a series of Swedish lakes by Graneli (1978).

Graneli, however, found that the oxygen consumption rate of sediments from cores did not respond to short-term increases in sedimented organic matter. He hypothesized that the oxygen uptake rate of sediments reflects a long-term integral of sedimented organic matter rather than short-term variation. Observations from the prairie lakes support this idea. Barica and Mathias (1979) found that the oxygen consumption rate per square metre of any lake was remarkably similar from year-to-year, despite the fact that there were 5-fold annual differences in average summer chlorophyll *a* in a number of lakes (Table 1), and considerable annual variation in primary production (unpublished data) and algal community structure (Kling 1975). It is clear that measurement of total primary production must be available for several years if winter oxygen depletion rates are to be predicted with any accuracy.

THE SEDIMENT AREA:LAKE VOLUME RATIO

The sediment area:lake volume ratio accounts for 72 and 78% of the variation in oxygen depletion rates shown in Fig. 2 (r^2 for regression = 0.72 and 0.78 for upper and lower lines, respectively). In addition, it differentiates between lakes which are classed by other measures as oligotrophic and eutrophic. The fact that we find two different mean oxygen consumption rates for lakes of different trophic state does not necessarily imply a strict dichotomy. Rather, oxygen consumption rates undoubtedly vary as a continuous function of lake productivity, as is suggested by pre- and post-fertilization oxygen depletion rates for lakes 261 and 227. Figure 2 implies that sediment oxygen consumption rates could be estimated more accurately with a model incorporating both the sediment area:lake volume ratio and an appropriate measure of trophic status.

The importance of the area of sediment surface which lies in contact with a unit volume of lake water was early recognized by Alsterberg (1927) and Hutchinson (1941). Alsterberg postulated that the degree of horizontal water movement in the hypolimnion of stratified lakes is high, and deduced an apparent relationship between the chemical character of each water layer and the area of sediment in contact with it at the edges. Hutchinson (1941) later demonstrated that the bicarbonate concentration of each water layer in the hypolimnion could be predicted from the sediment area:water volume ratio of that layer, assuming a constant bicarbonate flux from the sediments. Recently, Hesslein (1980) showed that the same type of prediction can be made for methane and ammonia distribution in a stratified lake.

Fee (1979) has further demonstrated the importance of the sediment area:water volume ratio to lake metabolism. He found that summer primary production in the epilimnion of a number of Canadian Shield lakes was directly correlated to the A/V ratio of the epilimnion. He suggested that because the sediments were a major site for remineralization of particulate material, nutrients would reenter the epilimnion in direct proportion to the sediment surface area in contact with the epilimnion, but would be diluted in proportion to

epilimnion volume. Since in small lakes there is a strong correlation between epilimnion A/V and whole-lake A/V, ($r = 0.99$ for the lakes studied by Fee), the question arises whether winter oxygen depletion rates of shield lakes are a function of A/V as shown by the lower line in Fig. 2, or are actually a function of the preceding summer's primary production which is in turn related to A/V. We cannot distinguish between these alternatives with our present data, although both are equally possible.

Water Column Respiration

As the mean depth of lakes increases, the ratio of sediment surface area to lake volume decreases. The Y-intercepts of both regression lines in Fig. 2 estimate the winter oxygen consumption rate of a unit volume of water in an infinitely large lake, where the area of contacting sediment has theoretically decreased to zero. This rate appears to be the same (ca. 0.01 $g \cdot m^{-3} \cdot d^{-1}$) for both groups of lakes, suggesting that the oxygen consumption of material derived from the water column varies little with productive status. Rather, it is the sediment oxygen consumption which contributes to the difference between productive and less productive lakes. The Y-intercepts in Fig. 2 are not significantly different from 0.0, however, so that the importance of the oxidation of substrates in the water column cannot be demonstrated with the data we have presented. We do not imply that the water column is not a site for respiration, for the oxidation of substrates like methane can be readily measured in the water column (Rudd and Hamilton 1978), and the biological oxygen demand (B.O.D.) of lake water is an accepted parameter. Rather, we suggest that the oxidation of sestonic or dissolved organic matter in the water column in situ seems less important than the oxidation of materials derived from the sediments.

Limiting Oxygen Concentration

If winter oxygen depletion in lakes is largely associated with sediment respiration, then the rate of mixing which delivers oxygen to the sediment surface might potentially limit the rate of oxygen depletion. There is no evidence from Fig. 1 and 2 to suggest that respiration rates were oxygen limited. However, for most of the Ontario, ELA, and Arctic lakes reported, mean dissolved oxygen never dropped below 3 $mg \cdot L^{-1}$, and as will be shown below, oxygen does not limit the respiration rate when the mean dissolved oxygen concentration (D.O.) is greater than about 3 $mg \cdot L^{-1}$. Data for prairie lakes (Fig. 1 and 2) were selected to include only the linear portion of the oxygen depletion curves, generally where D.O. was above 3.0 $mg \cdot L^{-1}$ (Barica and Mathias 1979). However, when average D.O. fell below 3.0 $mg \cdot L^{-1}$ in prairie lakes, the oxygen depletion rate characteristically declined as shown in Fig. 3. Some of the variation in Fig. 3 is due to lake-to-lake differences in the oxygen consumption rate (see Barica and Mathias 1979), but most is undoubtedly sampling error associated with measuring the oxygen concentration near to the sediment with conventional water samplers.

The shape of the scatter plot in Fig. 3 suggests that above a near-sediment D.O. of 3–4 $g \cdot m^{-3}$, the sediment respiration rate is relatively constant regardless of the availability of oxygen, whereas below 3 $g \cdot m^{-3}$ it is limited by the rate of supply of O_2 to the sediment. Welch (1974) reported that the respiration rate in shallow Meretta Lake declined when the mean lake oxygen concentration was less than 4 $mg \cdot L^{-1}$ and water 0.5 m from the sediments was nearly anoxic. Graneli (1978) has demonstrated a close relationship between oxygen saturation and sediment oxygen uptake in cores from two Swedish lakes. Similarly, Hargrave (1972) has shown that the oxygen depletion rate in the hypolimnion of Lake Esrom is constant at oxygen concentrations from 14 to 4 $mg \cdot L^{-1}$, but decreases by 60% when D.O. falls below about 4 $mg \cdot L^{-1}$. In sediment cores from Marion Lake, Hargrave (1969) found that stirring of the overlying water increased the respiration rate when the D.O. fell below 8 $mg \cdot L^{-1}$. At oxygen concentrations above 8 $mg \cdot L^{-1}$, stirring had no effect. The threshold value, 8 $mg \cdot L^{-1}$, was higher in Hargrave's experiments than that found in the prairie lakes probably because at 15°C the sediment cores consumed oxygen at a higher rate, 0.48 $g \cdot m^{-2} \cdot d^{-1}$, than did lake sediments in winter. When the oxygen concentration in ice-covered lakes falls to about 3 or 4 $mg \cdot L^{-1}$, the rate of eddy diffusion in these lakes must be insufficient to overcome the low oxygen concentration gradient above the sediment, and the flux rate of oxygen to the sediments declines. At this point, aerobic bacteria would presumably give way to sulphate and nitrite reducers, methane bacteria, and other anaerobic microorganisms.

Water Mixing

The rate of supply of oxygen to the sediments of a lake can be described by:

$$\text{Oxygen flux rate} = K \cdot (\text{Oxygen concentration gradient})$$
$$(g \cdot cm^{-2} \cdot s^{-1}) = K\,(g \cdot cm^{-3} \cdot cm^{-1})$$

where K ($10^{-3} \cdot cm^2 \cdot s^{-1}$) is a coefficient of eddy diffusion. It is possible to estimate K for the prairie lakes from the equation if it is assumed that:

a) the rate of oxygen supply to the sediments is the same as the oxygen depletion rate,
b) the concentration gradient is given by the oxygen concentration 30 cm above the sediment minus the oxygen concentration at the sediment surface, divided by 30,
c) the oxygen concentration at the sediment surface is 0.0 $g \cdot m^{-3}$.

Assumptions a) and c) are valid only when the oxygen depletion rate is limited by oxygen availability. Accordingly, the estimates of K shown below were calculated only for lakes where the near-sediment oxygen concentration fell below 3.0 $g \cdot m^{-3}$. (Calculations used data shown in Fig. 3 for which the oxygen concentration was less than 3.0 $g \cdot m^{-3}$.) It should be noted that the tabled values of K shown below are minimum values. To the extent that assumption (c) is incorrect, estimates of K will be larger than calculated.

Lake	Area (ha)	n	Eddy diffusion rate (10^{-3} $cm^2 \cdot s^{-1}$) $\bar{x}$	s
885	2.4	12	2.50	1.31
255	3.2	5	4.83	3.31
882	5.7	3	3.97	1.02
587	6.9	4	3.82	2.11
675	9.7	2	4.47	0.84
318	18.6	3	5.13	4.38
Mean			3.72	

Accurate measurements of vertical eddy diffusion rates under ice cover have not been made. However, the average values shown above are somewhat higher than values determined for the hypolimnion of a small stratified lake on the Canadian Shield. Quay et al. (1980) report vertical eddy diffusion rates of 1.7 ± 0.2 and $2.4 \pm 0.7 \times 10^{-3}$ $cm^2 \cdot s^{-1}$ measured by tritium diffusion and rates of $1.5–3.5 \times 10^{-3}$ $cm^2 \cdot s^{-1}$ measured by heat transport; and Hesslein (1980) reports values of $2.8–3.3 \times 10^{-3}$ $cm^2 \cdot s^{-1}$ measured from methane diffusion in lake 227 of the Experimental Lakes Area. The slightly higher diffusion rates found in prairie lakes could result from sediment-bound density currents, produced by sediment heat transfer as proposed by Mortimer and Mackereth (1958). Alternatively, they could result from density currents brought about by the diurnal pulse in solar radiation. Even weak density currents might reach the sediment surface in these very shallow lakes (B. Kenney, Freshwater Institute, Winnipeg, Man., personal communication).

Winter observations in prairie lakes suggest that the eddy diffusion rates calculated from vertical oxygen data result from horizontal, rather than vertical, mixing processes. A slight but persistent oxygen gradient from beneath the ice to the lake bottom in prairie lakes (Fig. 4) indicates that vertical mixing is not complete during oxygen depletion. At the same time, successive depth profiles of oxygen concentration remain parallel, suggesting that deeper water layers are not depleted of oxygen at a faster rate than surface layers, as one might expect if oxygen were diffusing vertically to the sediments. Patterns of O_2 depletion in the hypolimnion of other lakes during summer stratification, for example Lake Windermere (Lund et al. 1963), similarly show decreases in oxygen independent of depth. Likens and Hasler (1962) and Likens and Ragotzkie (1966) by injecting radioactive ^{24}Na into ice-covered lakes, showed that horizontal diffusion was from one to three orders of magnitude higher than vertical diffusion. All of the above observations are consistent with the hypothesis that horizontal diffusion is more important than vertical diffusion in supplying oxygen to the littoral and sublittoral sediments in lakes.

The average eddy diffusion rate calculated above, 3.72×10^{-3} $cm^2 \cdot s^{-1}$, is sufficient to deliver oxygen to the sediments at a rate of 0.23 $g \cdot O_2 \cdot m^{-2} \cdot d^{-1}$ (mean oxygen consumption rate for the eutrophic lakes in Fig. 2), so long as there is about 2.1 $g \cdot m^{-3}$ of oxygen, 30 cm above the sediment surface. In prairie lakes, because there is always a slight oxygen concentration gradient from the ice to the sediment surface (e.g. Fig. 4b), a concentration of 2.1 $g \cdot m^{-3}$ 30 cm above the sediment surface corresponds to a whole-lake average concentration of 3.8 $g \cdot m^{-3}$. For most of the winter, oxygen concentrations are well in excess of 3.8 $g \cdot m^{-3}$, and oxygen supply cannot be limiting the respiration rate. At high oxygen concentrations, therefore, some other process appears to limit sediment respiration, most likely the rate of diffusion of oxidizable substrate upward within the sediment to the oxidized microzone.

Implications for Lake Management

The rate of winter oxygen depletion can be used to indicate the trophic status of lakes. However, since oxygen depletion rates are also influenced by the shape of lakes, the "depletion rate-trophic state" relationship can best be seen after the effects of basin morphometry are separated out explicitly by regression analysis as in Fig. 2. Further work is required to establish a quantitative relation between winter oxygen depletion and annual primary production.

The usefulness of this A/V ratio in clarifying relationships among the oxygen depletion rates of lakes suggests that the same procedure be applied when examining hypolimnetic oxygen deficits. Since stable thermoclines are effective barriers to the vertical transport of oxygen, the hypolimnion of a lake is essentially a closed oxygen system as is an ice-covered lake. The sediment area:water volume ratio would then be calculated for the hypolimnion alone.

When the mean dissolved oxygen concentration in an ice-covered prairie lake is greater than about 3.8 $mg \cdot L^{-1}$, increasing the oxygen supply to the sediments, either by mixing or by increasing the D.O. concentration, should have no effect on the rate of oxygen consumption. When lakes are approaching winterkill conditions, however, with average D.O. concentrations below about 3.8 $mg \cdot L^{-1}$, water mixing beneath an intact ice cover should effectively hasten the onset of anoxia.

The rate of winter oxygen depletion appears to be less dependent on the oxidation of materials residing in the water column than on material from the sedi-

ments. Moreover, the "water column" component of whole-lake oxygen consumption is the same for oligotrophic and eutrophic lakes, despite higher winter concentrations of dissolved organic carbon in the latter. This suggests that addition of dissolved organic substrates to the water column may not substantially increase the rate of oxygen consumption in ice-covered lakes. This prediction has been partially tested by an experiment in which the introduction of dissolved methane into an ice-covered lake brought about no measurable increase in whole-lake oxygen consumption. The lake was well mixed by this introduction, and average D.O. was well above 10 $mg \cdot L^{-1}$ during the experiment (H. Welch unpublished data).

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Distribution of Fatty Acids in Lipids of the Common Atlantic Sea Urchin *Strongylocentrotus droebachiensis*

T. TAKAGI,[1] C. A. EATON,[2] AND R. G. ACKMAN[3]

Department of Fisheries and Oceans, Technology Branch, Halifax, N.S. B3J 2S7

TAKAGI, T., C. A. EATON, AND R. G. ACKMAN. 1980. Distribution of fatty acids in lipids of the common Atlantic sea urchin *Strongylocentrotus droebachiensis*. Can. J. Fish. Aquat. Sci. 37: 195–202.

Lipid classes and fatty acid details of sea urchins *Strongylocentrotus droebachiensis* harvested in subtidal water near Halifax, and in deep water in St. Margaret's Bay, were compared. All samples contained an eicosenoic acid with 5,6-ethylenic unsaturation and two non-methylene-interrupted eicosadienoic acids (NMID) with the one 5,6 bond and a second in either the 11,12 or 13,14 positions. Structures were identified by combinations of ozonolysis, partial hydrazine reduction and argentation-TLC and by mass spectra of pyrrolidine derivatives. No 3,11-eicosadienoic acid was observed and elongation of C_{20} materials with few ethylenic bonds to C_{22} homologues was confined to 11–20:1 and to the minor 5,13–20:2 NMID and did not include either the relatively important 5–20:1 monoene or the 5,11–20:2 NMID. Individual animals showed considerable variation in total lipid and lipid classes, but the consistent distribution of the fatty acids with 5,6 unsaturation, irrespective of the nutritional status of the animal, suggests a basic role in membrane function.

Key words: Strongylocentrotus droebachiensis, sea urchin lipids, sea urchin fatty acids, non-methylene-interrupted fatty acids, eicosadienoic fatty acids

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Nous avons comparé les classes de lipides et les détails d'acides gras d'oursins communs, *Strongylocentrotus droebrachiensis*, récoltés dans les eaux subtidales près d'Halifax et en eaux profondes dans la baie St. Margaret. Tous les échantillons contiennent un acide éicosènoïque à non-saturation 5,6 éthylénique et deux acides eicosadiènoïques NMID (« non-methylene-interrupted ») avec cette liaison 5,6 et une seconde, soit en position 11, 12, soit 13, 14. Les structures furent identifiées par combinaisons d'ozonolyse, réduction partielle par hydrazine et argentation-TLC et par spectres de masse des dérivés de pyrrolidine. On n'a pas observé d'acide 3,11 eicosadiènoïque, et l'élongation de matériaux C_{20} avec peu de liaisons éthyléniques en des homologues C_{22} est limitée à 11–20:1 et au 5,13–20:2 NMID relativement peu important; elle n'inclut ni le 5–20:1 monoène ni le 5,11–20:2 NMID relativement importants. Il y a grande variation d'un animal à l'autre des lipides totaux et des classes de lipides, mais la répartition uniforme des acides gras avec non-saturation 5,6, sans égard à la condition alimentaire de l'animal, suggère un rôle fondamental pour la fonction de la membrane.

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THE impact of common sea urchins in destroying kelp and other seaweed beds important for the reproduction and support of valuable species of fish and invertebrates such as lobster is a matter of considerable concern on the Atlantic coast of Canada (Mann and Breen 1972; Miller and Mann 1973; Breen and Mann 1976; Evans and Mann 1977). Sea urchins can endure rather long periods without apparent sources of nutriment and lipids are the primary storage materials for their energy during fasting (Ferguson 1969). On the other hand, sea urchins are also an essential intermediate in the energy flow from seaweeds to the commercially important crustacea and other animals, and lipids are presumed to play an important role in the flow. Most fatty acids of sea urchins and other echinoderm lipids in the literature are identified only by a shorthand notation indicating the chain length and numbers of ethylenic bonds (Allen 1968; Fujino et al. 1970; Miwa 1975; Chelomin and Svetashev 1978; Kozhina et al. 1978; Metzman et al. 1978). Recently, Kochi reported

[1]Present address: University of Hokkaido, Hokkaido, Hakodate, Japan.

[2]Present address: Department of Fisheries and Oceans, P.O. Box 550, Halifax, N.S. B3J 2S7.

[3]Present address: Nova Scotia Technical College, Fisheries Research and Technology Laboratory, P.O. Box 1000, Halifax, N.S. B3J 2X4.

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the occurrence of 3,11- and 5,11-eicosadienoic (20:2) acids in the roe lipids of sea urchins, *Strongylocentrotus pulcherrimus* and *S. crassispina*, obtained respectively from Japan and Korea (1976a, b, c, d). We decided to investigate the composition of lipids in the common Atlantic sea urchin *Strongylocentrotus droebachiensis* in some detail, and with the recent augmentation in knowledge of the fatty acids of major local seaweeds (Ackman and McLachlan 1977) indicating the absence of C_{20} non-methylene-interrupted dienoic (NMID) fatty acids, it also became possible to investigate NMID in relation to food chain transfer (Paradis and Ackman 1977). To achieve these dual objectives sea urchins from two different habitats near Halifax were compared. The lipids differed appreciably, but the fatty acids showed only a few points of difference. The structures of all C_{20} monoethylenic and NMID fatty acids were determined in detail. These were qualitatively and quantitatively similar in all samples of sea urchins, suggesting an endogenous origin rather than a food chain origin. No 3,11-eicosadienoic acid was found.

Experimental Procedures

Sea urchins for this study were collected at low tide from Herring Cove near Halifax, N.S. on June 18 and 23, 1978, and at St. Margarets Bay on September 21, 1978 from a depth of 8 m. They were frozen and temporarily stored at −20°C. The shell contents (viscera, gonads, etc.) were extracted by the method of Bligh and Dyer (1959). Lipid class analyses were carried out by Iatroscan TH-10 Mark II Analyzer (Iatron Laboratories Inc., Tokyo) by procedures described elsewhere (Sipos and Ackman 1978). Fractionation of the total lipids into polar and neutral lipids was carried out by column chromatography using silicic acid (Malinkrodt, for chromatographic use) with chloroform and methanol as the solvents. Lipids were saponified and unsaponifiables were removed by procedure Ca-6b-53 of the AOCS (1972). Following acidification with dilute HCl, fatty acids were recovered by petroleum ether extraction and were converted to methyl esters by heating in a sealed tube at 100°C for 10 min with 7% BF_3–MeOH. A nitrogen atmosphere was maintained at all times. Gas–liquid chromatography (GLC) of methyl esters was carried out with a Perkin-Elmer model 910 chromatograph equipped with a flame ionization detector. The stainless-steel wall-coated open-tubular columns used were 47 m in length × 0.25 mm ID, coated with SILAR-5CP or SILAR-7CP (Applied Science Laboratories, State College, PA). The columns were operated isothermally at 180°C. Helium was used as a carrier gas at 50 psig. Injector, detector, and manifold temperatures were maintained at 260°C. Other operating details are given elsewhere (Ackman and Hooper 1973).

Fatty acid methyl esters of the C_{20} chain length were separated from the total methyl esters by preparative GLC using a packed stainless steel column (10% diethyleneglycol-succinate polyester on Chromosorb W, 1.5 m in length × 6 mm ID, carrier gas He, column temperature 190°C), employing a thermal conductivity detector, a heated collection unit (250°C), and a glass collection tube. The separation of recovered methyl esters on the basis of degree of unsaturation was carried out by preparative argentation TLC on silica gel plates (Prekotes, Adsorbosil 5, Applied Science Laboratories, impregnated by dipping in 10% silver nitrate in acetonitrile solution). Development was carried out with benzene: *n*-hexane::2:1 and the lipid bands were visualized by spraying with 2′,7′-dichlorofluorescein-ethanol solution followed by examination under ultraviolet light. The silicic acid was transferred to a screw cap centrifuge tube and extracted successively with 10-mL volumes of hexane, hexane:$CHCl_3$ 1:1, and $CHCl_3$.

The oxidative ozonolysis of methyl esters was carried out in BF_3–MeOH medium by procedures recently reported (Ackman 1977; Sebedio and Ackman 1978). Methyl esters (3–7 mg) and 2 mL of 7% BF_3–MeOH were placed in a 10-mL screwcap centrifuge tube, and ozone in oxygen (from a Welsbach Model T-408 ozone generator, Welsbach Co., PA) was bubbled in at a flow rate of 250 mL/min for 1 min. The capped tube was heated at 100°C for 1 h, water (2 mL) was added and the products were extracted twice with $CHCl_3$ (2 mL each time). After washing the $CHCl_3$ combined extracts with water (5 mL) and gently concentrating the solvent, the methyl esters obtained were subjected to GLC analysis. A stainless-steel wall-coated open-tubular column (47 m in length × 0.25 mm ID) coated with SILAR-5CP and operated at 150°C was used for dicarboxylic acid esters and long-chain monocarboxylic acid esters. A similar butanediolsuccinate polyester column was operated at 80°C for short-chain monocarboxylic acid esters.

Partial hydrogenation of polyethylenic acids was carried out with 95% hydrazine (Eastman Organic Chemicals, Rochester, NY) by established procedures (Dutton et al. 1963). The mixture of 10% (v/v) hydrazine in MeOH (10 mL) and fatty acid (10–15 mg) was stirred in air at 50–55°C, with a Teflon-coated magnetic stirrer bar, for 0.8–1.0 h. The product mixture was acidified with dilute HCl, extracted with *n*-hexane, and converted to methyl esters with 7% BF_3–MeOH, followed by analysis on SILAR-5CP.

The pyrrolidides of fatty acids were prepared on a microscale by the method of Andersson et al. (1975). Methyl esters (10 mg) were dissolved in freshly distilled pyrrolidine (1 mL) and acetic acid (0.1 mL). The mixture was held at 100°C in a screw-cap centrifuge tube for 0.5 h under a nitrogen atmosphere. After addition of water (3 mL) the products were extracted twice with methylene chloride (3 mL). This solution was washed with dilute HCl and water until neutral. After drying over anhydrous magnesium sulfate, the solvent was removed by nitrogen jet. The conversion was checked by GLC on a glass column 3 m in length × 2 mm ID packed with 3% OV-1 on Chromosorb W (80–100 mesh). The mass spectra of the pyrrolidides were obtained on a Finnigan 4000 combined gas chromatograph-mass spectrometer operated at an ionization potential of 70 eV. The hexane solution of the pyrrolidides was injected on a glass column (1.8 m in length × 3 mm ID) packed with 3% OV-17 on Chromosorb W (80–100 mesh). Helium was used as the carrier gas at a flow rate of 20 mL/min. The temperatures of column, separator, transfer line, and ion source were 230, 250, 222, and 250°C, respectively.

Results and Discussion

Table 1 shows total lipid recovery and lipid class compositions for pooled groups of sea urchins; Table 2

TABLE 1. Sample data and lipid recovery from pooled sea urchin samples with detailed lipid class composition and recovery of fatty acids from lipids as methyl esters.

Collection data				
Place	Herring Cove		St. Margaret's Bay	
Date	June 1978		September 1978	
Sample no.[a]	1	2	3	4
Number of urchins	27	11	15	31
Whole animal weight range (g)	2.3–83.8	20.0–29.8	4.1–13.2	9.4–48.3
Whole animal average weight (g)	17.8	23.4	10.4	21.4
Lipid data[b]				
Recovery of lipid shell contents (w/w%)	1.78	1.94	0.21	0.69
Phospholipids[c]	51.1	52.8	76.4	75.4
Diglycerides	—	—	3.3	—
Sterols	4.5	5.7	11.9	13.5
Triglycerides	37.3	39.1	7.0	5.4
Diacylglyceryl ethers	0.3	0.6	—	1.0
Free fatty acids	6.5	1.7	—	3.2
Sterol esters	0.6	0.6	1.0	0.1
Hydrocarbons	1.1	0.9	0.3	0.3
Yield of methyl esters from total lipids (w/w%)	48	47	35	34

[a]Samples No. 2 and No. 3 appear in Table 3 as No. 1 and No. 6.
[b]Classes determined by Iatroscan analysis.
[c]This fraction includes some pigments and other polar compounds.

lipid recovery for individual animals selected for similarity in weight. Total lipids and triglyceride contents were both definitely lower in St. Margaret's Bay sea urchins. The total lipid contents of the sea urchins collected in one place can differ by a factor of up to three within each of the two geographical locations, but the overall difference by location is approximately fourfold and thus much more than would be expected from prespawning and postspawning condition.

Table 3 shows the detailed fatty acid compositions of various sea urchin lipids. These include the saturated, monoethylenic, and all-*cis*, methylene-interrupted polyethylenic fatty acids usual for marine lipids. The identification of these component peaks rests on comparison with literature retention data and mixed injections with fatty acid methyl esters prepared from capelin (*Mallotus villosus*) oil (Eaton et al. 1975), oyster *Crassostrea virginica* lipids (Watanabe and Ackman 1974; Paradis and Ackman 1975, 1977), and Δ5–20:1 from the seed oil of *Limnanthes douglasii* (Phillips et al. 1971). Reference samples of authentic *cis*-9-18:1, *cis*-11-20:1, and *cis*-13-22:1 were also employed. The fractions of methyl esters of sea urchin fatty acids separated by their degrees of unsaturation with $AgNO_3$–TLC were also used for identifications and to verify that specific peaks in gas–liquid chromatographic analyses of total esters were single components with one or more *cis* ethylenic bonds.

It is noteworthy that the content of *cis*-5-20:1 (20:ω15) is as much as 11% of total fatty acids. The occurrence of this acid in marine animals has been reported in periwinkle *Littorina littorea*, moon snail *Lunata triseriata*, and sand shrimp *Crangon septemspinosus* (Ackman and Hooper 1973), but at much lower levels ($\leq$0.2%). It is not commonly reported as a fatty acid typical of marine oils or animal tissue lipids. The occurrence of 20:2 NMID isomers has been reported in several marine animals, e.g. 5,11 and 5,13

TABLE 2. Lipid recovery from selected individual sea urchins and recovery of fatty acids from lipids as methyl esters.

Place collected	Herring Cove				St. Margaret's Bay			
Sample number[a]	2	3	4	Average	7	8	9	Average
Weight whole animal (g)	25.9	25.3	28.7	26.6	30.5	30.3	29.2	30.0
Weight shell (g)	11.2[b]	9.5[b]	9.8[b]	10.2[b]	8.7[c]	7.2[c]	8.0[c]	8.0[c]
Weight shell contents (g)	14.7	15.8	18.9	16.4[d]	17.5	18.5	17.2	17.7[e]
Recovery of lipids from shell contents (w/w%)	1.46	1.69	2.45	1.86	0.43	0.48	0.46	0.46
Yield of methyl esters from total lipids (w/w%)	52	50	56	53	44	40	42	42

[a]Sample numbers are the same as those in Table 3.
[b]Wet weight, average 38% of whole animal wet weight.
[c]Dried for 1 h at 110°C, average 27% of whole animal wet weight.
[d]Averages give 62% of whole animal.
[e]Averages give 59% of whole animal.

TABLE 3. Composition (w/w%) of fatty acids recovered from different samples of sea urchin lipids.

Place collected	Herring Cove						St. Margaret's Bay					
Sample number	1	2	3	4	5	5	6	7	8	9	10	10
Lipid[a]	TL	TL	TL	TL	NL	PL	TL	TL	TL	TL	NL	PL
Fatty acid[b]												
14:0	7.3	11.3	9.8	9.6	10.4	10.0	7.2	7.0	4.1	4.5	11.5	8.1
15:0	0.5	0.4	0.4	0.2	0.7	0.5	0.9	1.2	0.5	0.7	2.5	1.4
16:0	9.8	11.8	11.7	10.8	12.9	9.7	15.8	15.0	5.7	11.4	16.1	15.4
17:0	—	—	0.1	0.2	0.2	—	0.5	0.5	0.2	0.1	—	0.7
18:0	2.4	2.1	2.0	1.8	2.4	2.6	3.7	4.1	3.7	3.4	7.3	6.0
20:0	0.6	0.5	0.9	0.8	1.9	0.8	0.4	0.3	1.1	0.4	0.4	0.3
Σ	20.6	26.1	24.8	23.3	28.5	23.6	28.5	28.7	15.2	20.4	37.8	31.9
14:1ω5	1.1	1.3	1.0	0.7	3.2	1.1	0.9	0.6	0.4	0.4	2.1	1.8
16:1ω11	—	0.7	0.2	0.1	2.1	2.2	0.2	0.1	0.8	0.2	—	—
16:1ω9	0.9	0.2	0.1	tr	0.9	0.1	—	0.3	0.3	0.1	—	—
16:1ω7	3.9	3.8	3.8	4.0	5.5	4.6	8.5[c]	4.7	1.9	3.7	8.4[c]	9.8[c]
16:1ω5	1.9	1.9	2.3	2.3	1.3	2.6	—	1.2	0.8	1.4	—	—
18:1ω13	0.4	0.5	0.7	0.4	0.3	0.6	0.3	0.7	0.8	0.6	0.2	—
18:1ω9	2.6	3.8	2.7	1.8	1.1	1.9	2.8	2.7	3.7	1.2	5.4	3.3
18:1ω7	2.4	2.2	2.5	3.1	2.0	3.4	3.1	3.0	5.6	3.7	5.9	2.7
20:1ω15	4.2	4.3	6.5	5.2	4.2	5.7	6.0	7.8	9.9	11.1	4.2	3.2
20:1ω11	0.4	0.6	0.7	0.6	1.1	0.6	0.3	0.4	0.6	0.9	0.3	0.2
20:1ω9	3.4	2.3	3.6	3.8	3.6	4.2	2.2	2.2	6.4	2.9	1.9	0.9
20:1ω7	0.8	0.6	1.0	1.4	2.4	1.4	0.6	0.8	1.6	1.2	0.7	0.3
22:1ω11	1.1	1.1	1.8	2.0	2.9	2.4	0.6	0.5	1.8	0.8	0.2	—
Σ	22.2	23.3	26.9	25.4	30.6	28.8	25.3	25.1	33.6	27.9	29.3	22.2
18:2ω6	1.9	3.3	1.9	1.0	0.8	1.4	1.8	2.0	1.8	0.9	3.3	0.9
20:2Δ5, 11	4.3	4.2	6.3	5.6	5.3	6.3	3.8	3.9	8.3	5.4	2.6	2.2
20:2Δ5, 13	1.2	1.0	2.2	1.9	2.3	1.8	1.0	1.0	1.6	3.2	0.6	0.5
20:2ω6	1.8	1.4	2.0	1.7	1.5	1.9	1.7	1.8	2.6	2.2	1.1	0.4
22:2Δ7, 13	—	0.2	0.3	0.1	0.7	0.1	—	—	—	—	—	—
22:2Δ7, 15	0.3	0.5	1.0	0.7	1.2	0.7	1.5	1.2	1.5	3.2	0.3	—
Σ	9.7	10.6	13.7	11.1	11.8	12.2	9.8	9.9	15.8	14.9	7.9	4.0
18:3ω6	0.3	0.8	0.5	0.6	0.4	0.4	0.5	0.9	0.9	0.7	?	?
18:3ω3	2.9	3.4	2.2	1.2	1.3	1.9	0.4	0.8	1.0	—	2.0	0.3
20:3ω6	0.2	0.2	0.2	0.2	0.1	0.1	0.1	—	—	0.1	—	—
20:3ω3	2.5	1.3	2.0	2.1	1.1	2.0	0.6	0.5	1.1	0.7	0.5	0.3
Σ	5.9	5.7	4.9	4.1	2.9	4.4	1.6	2.2	3.0	1.4	2.5	0.6
18:4ω3	8.4	8.2	5.3	4.7	2.7	4.7	0.9	0.9	1.0	0.6	3.7	0.7
20:4ω6	8.8	8.7	8.1	6.8	7.2	6.5	16.3	16.6	16.8	16.9	6.6	16.1
20:4ω3	1.7	0.9	1.2	1.4	0.6	1.3	—	0.4	—	—	—	—
Σ	18.9	17.8	14.6	12.9	10.5	12.5	17.2	17.5	17.8	17.5	10.3	16.8
20:5ω3	22.6	16.8	15.0	21.4	14.7	17.7	17.3	16.3	13.9	17.6	10.4	22.9
22:5ω3	0.1	0.1	0.1	0.5	1.2	0.4	—	—	tr	—	—	—
Σ	22.7	16.9	15.1	21.9	14.9	18.1	17.3	16.3	13.9	17.6	10.4	22.9
22:6ω3	0.7	0.4	0.4	1.5	0.8	0.9	0.5	—	0.8	0.2	1.9	1.7

[a]Lipids 1 and 6 are from pooled samples, 2–5 and 7–10 from single animals. TL = Total lipid, NL = Neutral lipid, PL = Polar lipid.

[b]Iso and Anteiso $C_{15:0}$ and $C_{17:0}$ and C_{16} polyethylenic acids were present in minor amounts.

[c]Total 16:1 — detail lost through recorder overload.

TABLE 4. Mono and dicarbonylic esetrs (as mole ratios) from the ozonolysis of standards and sea urchin lipids, supporting the assigned double bond positions of the C_{20} non-methylene interrupted dienes.

O_3 product	Standards		Sea urchins		
	Δ9-18:1	Δ11-20:1	Δ5-20:1	NMID[a] mixture	Monoene[b] mixture
M 7	—	—	—	4.9	trace
M 9	46.1	49.3	—	23.5	23.9
M 15	—	—	50.3	—	24.7
D 5	—	—	49.7	35.1	24.5
D 6	—	—	—	32.3	—
D 8	—	—	—	4.2	—
D 9	53.9	—	—	—	—
D 11	—	50.1	—	—	24.0
D 13	—	—	—	—	2.9

[a]C_{20} NMID fraction isolated by preparative GLC followed by $AgNO_3$–TLC.

[b]20:1 isomers isolated by $AgNO_3$–TLC from the partial hydrazine reduction of fraction a.

isomers in periwinkle, moon snail, and sand shrimp (Ackman and Hooper 1973), 5,11 and 5,13 and 5,15 in oyster *C. virginica* (Paradis and Ackman 1975), and generally in species as diverse as Mexican Ridley turtle, jellyfish, mussels, scallops, quahaug, starfish, and sturgeon (Paradis and Ackman 1977). In these, 20:2 NMID was 1.71% in total methyl esters of oyster *C. virginica*, while the starfish *Asterias vulgaris* had the highest reported content at 5.6%. Allen (1968) reported 6.2–6.5% of 8,11-20:2 in the sea urchin *Echinus esculentus*, but this GLC peak was very likely made up of several 20:2 NMID such as we report herein. Typically methyl esters from total lipids of sea urchins *S. droebachiensis* examined in this study showed as much as 5–10% total 20:2 NMID.

The structures of the *cis*-5-20:1 (20:1ω15), 5,11- and 5,13-20:2 have each been confirmed by the oxidative ozonolysis of the methyl esters and through reexamination of the monoethylenic isomers produced by the partial hydrazine reduction of 20:2 NMID and by comparison with authentic 9-18:1 and 11-20:1 as reference compounds. The products are shown in Table 4. The 20:2 fraction was concentrated by preparative GLC of total methyl esters. Argentation-TLC of the fraction gave five bands identified as: 6,9,12,15-18:4, R_f 0.04 (from chain length overlap on the DEGS preparative GLC column); 20:2 NMID, R_f 0.19; 11,14-20:2 (20:2ω6),[4] R_f 0.29; 5-20:1, R_f 0.50; and 11-20:1, R_f 0.58. The 20:2 NMID showed only two major components in a ratio of approximately 5:1. The mole ratios of the oxidation products (Table 4) from

[4]Biochemically "normal" fatty acids with methylene-interrupted structures are conveniently denoted by chain length: number of *cis* ethylenic bonds and *ωn* where *n* gives the position of the ethylenic bond closest to the terminal methyl group.

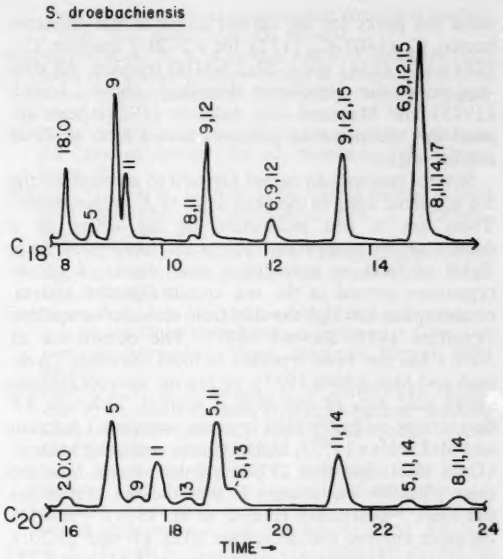

FIG. 1. Parts of a gas–liquid chromatographic record from analysis of fatty acids in *S. droebachiensis*. Numbers above lines refer to positions of ethylenic bonds counted from carboxyl group and numbers below lines to adjusted retention time in minutes. There were no attenuation changes. Liquid phase, SILAR-5CP; column 47 m × 0.25 mm ID, wall-coated, stainless steel.

the 20:2 NMID fatty acid mixture can be explained as follows: (D6, M9) and (D8, M7) were produced, respectively, from the central and terminal parts of 5,11- and 5,13-20:2 and D5 from both. Recovery of the hydrazine hydrogenation products from the mixture of 5,11- and 5,13-20:2 showed the following composition: 20:0, 62.0%; 5-20:1, 12.7%; 11-20:1, 10.8%; 13-20:1, 1.9% and the original 20:2 mixture 71.6%. This data supports the hydrogenation of each double bond at an equal rate, since the sum of 11- and 13-20:1 corresponds to 5-20:1 content. The ozonolysis of the total monoene fraction (5-20:1, 49.6%; 11-20:1, 41.7%; 13-20:1, 8.7%), isolated from the reduction mixture by $AgNO_3$–TLC, gave the products also shown in Table 4. Each pair of M9, D11; M15, D5; and M7, D13 confirmed the formation of 5-,11-, and 13-20:1 by the hydrogenation. Particular attention was paid to the identification of M17 but it could not be detected in any ozonolysis product. There is little doubt that in the total lipids of *S. droebachiensis* the main components for 20:2 NMID are 5,11- and 5,13-20:2 but the 3,11-20:2 reported by Kochi (1976a, b, c, d) could not be found.

The mass spectra of the pyrrolidides should show (Andersson et al. 1975; Valicenti et al. 1978) intervals of mass number 12 between the maxima in the frag-

ment ion peaks for the carbon atoms of the ethylenic bonds: C_4 (140)–C_5 (152) for a 5-20:1 fraction, C_{10} (224)–C_{11} (234) for a 20:2 NMID fraction. All data supported the structures described above. Joseph (1975) and Mayzaud and Ackman (1978) have applied this technique to polyunsaturated fatty acids of marine origin.

Several reasons can be put forward to account for the 5,6 ethylenic bond in the fatty acids of *S. drebachiensis*. These are in situ production by the action of a desaturase on preexisting fatty acids, absorption from lipids of food or assimilation from lipids of microorganisms normal in the sea urchin digestive system, or absorption through the skin from seawater or epiflora (Pavillon 1977; Stewart 1977). The occurrence of 5-20:1 has not been reported in local seaweeds (Ackman and McLachlan 1977), or marine microorganisms (fatty acid data of any type is scarce), although 5,6 desaturases evidently exist in some seaweeds (Ackman and McLachlan 1977), higher plants, and some bacteria (Gurr 1974; Sprecher 1977). At the present time the most plausible explanation is that the sea urchin has the basic 5-desaturase (Ninno et al. 1974). Probably the same enzyme will desaturate 20:0, 11- and 13-20:1 in common, because the proportions of 5,11- and 5,13-20:2 NMID very closely follow those of the 11- and 13-20:1 isomers (Fig. 1). Two-carbon chain elongation of 11-20:1, 5,11-20:2, and 5,13-20:2 produces components tentatively identified as 13-22:1, 7,13-, and 7,15-22:2, respectively. However, the elongation product of 9-20:1, namely 11-22:1, could not be detected and the 7,13-22:2 produced from 5,11-20:2 was a minor component. Evidently this elongation occurs selectively.

The analysis of total lipids of marine organisms for fatty acids can be uninformative if there is much difference in details of the respective fatty acid compositions of triglycerides and phospholipids, since the proportions of these lipids are often variable. In some studies only a limited number of fatty acids are quantitatively important enough to be examined seriously (Lambertsen 1978), but the rest can provide valuable corroborative detail. Individual lipid components (Table 2) of two sea urchin samples, as well as pooled lipids (Table 1) were examined for fatty acids (Table 3). The 16:0 and 16:1ω7, and total 18:1, are higher in neutral lipid, while 20:5ω3 is distinctly higher in polar lipid. Among other fatty acids of special interest, 14:0, 18:0, 20:1, 18:2ω6, 18:3ω3, 18:4ω3, 20:4ω6, and 22:6 are not more important in the one lipid than in other. The sea urchin viscera examined by Kochi (1976a, b, c, d) support these generalities for 16:0, 16:1, 18:1, and 20:5ω3. It is thus possible to speculate that the 16:0, 16:1ω7, and 18:1ω7 (especially the latter) are synthesized de novo by the sea urchin and stored as energy sources. Although all three acids are available from seaweeds (Ackman and McLachlan 1977), the high proportion of 18:1ω7 relative to 18:1ω9 is an important indicator of the role of de novo 16:0 since the biosynthetic pathway is 16:0 → 16:1ω7 → 18:1ω7.

Many seaweeds also contain 20:5ω3, but more generally the sea urchin does not accumulate the typical seaweed precursor acid 18:3ω3, and this suggests that ingested 18:3ω3 could partly be used for biosynthesis of 20:5ω3. This fatty acid is found in phospholipids which are structurally parts of membranes. The St. Margaret's Bay samples were lower in total lipid (Tables 1 and 2) and accordingly the lipid includes relatively more phospholipids. In these samples 20:4ω6 was at least twice as high a percentage of total fatty acids as for the Herring Cove samples, but none of the precursor acids (e.g. 18:2ω6, 18:3ω6, 20:3ω6) were obviously enhanced. The St. Margaret's Bay sea urchins were collected from beds of *Laminaria digitata*, a seaweed known to have 20:4ω6 almost equal to 20:5ω3 (respectively, 11 and 14% in the study of Ackman and McLachlan 1977), whereas the Herring Cove sea urchins came from the varigated growths of the subtidal zone. This suggests that in sea urchins which feed on seaweeds where linoleic-type fatty acids (e.g. 18:2ω6, 20:4ω6) may be plentiful (Ackman and McLachlan 1977) membranes may partly substitute this longer chain fatty acid (20:4ω6) of the linoleic family for 20:5ω3. Sea urchin eggs from two species (*Strongylocentrotus intermedius* and *Arbacia punctulata*) are reported to have phospholipids with 20:4ω6 ~ 20:5ω3 (Chelomin and Svetashev 1978; Kozhina et al. 1978; Metzman et al. 1978). Allen (1968) found the same to be true of lipids from various parts of three different echinoderms (*Asterias rubens, Echinus esculentus*, and *Holothuria forskali*), and Kochi (1976d) reported relatively equal 20:3ω3 (putatively 20:4ω6) and 20:5ω3 in gonads of *Strongylocentrotus pulcherrimus, Anthocidaris crassispina*, and *Pseudocentrotus depressus*, with somewhat less 20:4ω6 in *Strongylocentrotus nudus*. The ability to accumulate a high proportion of 20:4ω6 may thus be a property of echinoderms and sets them apart from filter-feeding bivalve molluscs (Ackman et al. 1974; Watanabe and Ackman 1974) as well as gastropods (e.g. *L. littorea*) also grazing on seaweed, or carnivorous molluscs such as *L. triseriata* (Ackman and Hooper 1973).

As sea urchins spawn in summer the status of gonad development should not affect the results we have obtained for St. Margaret's Bay animals and the lipid should be basic to the animal body itself (Giese 1966). The low level of triglycerides in the lipid of this lot of samples (Table 1) suggests that these animals, which are known to be part of very dense populations (Breen and Mann 1976), are at a nutritional disadvantage compared to the Herring Cove samples. The conservation of 20:4ω6 is therefore additional support for it having a functional role.

In a previous report (Ackman and Hooper 1973) on 20:2 NMID it was proposed that the NMID could substitute in part for polyunsaturated fatty acids such

as 20:4ω6 because of the similarity of the ethylenic bond position closest to the terminal methyl group. Clear evidence for this is not forthcoming in the fatty acid details of Table 3. The alternative explanation advanced earlier, namely that the 5,6 ethylenic bond confers on the nearby ester linkage resistance to hydrolysis by bacteria or other organisms, and therefore contributes stability to membranes exposed freely to seawater, may be more likely since structurally similar, although longer, fatty acids are now known to be present in marine sponges (Litchfield and Marcantonio 1978). However the fact that these unusual fatty acids are more or less a constant proportion of fatty acids in sea urchins (Table 3) argues that they have some role common to all tissue.

Sea urchins are increasingly used in biological research. The change in the lipids during embryonic development continues to attract much attention (Öhman 1944; Chelomin and Svetashev 1978; Kozhina et al. 1978; Metzman et al. 1978). This could be particularly useful point to examine the role of NMID, but to date we are unaware of suitable analytical technology being applied to these lipids.

Sea urchins are easily collected for food purposes, although they are mainly so used in Japan, especially in a pickled or salt-cured form (Fujino et al. 1970; Miwa 1975). Our results for St. Margaret's Bay suggest that the dense population is not well nourished. The variability which we observed in lipids of local sea urchins therefore indicates that the composition of the shell contents should be more closely examined to establish nutritional parameters if they are to be used for edible purposes.

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Growth of Underyearling Sockeye Salmon (*Oncorhynchus nerka*) under Constant and Cyclic Temperatures in Relation to Live Zooplankton Ration Size[1]

R. M. BIETTE[2] AND G. H. GEEN

Department of Biological Sciences, Simon Fraser University, Burnaby, B.C. V5A 1S6

BIETTE, R. M., AND G. H. GEEN. 1980. Growth of underyearling salmon (*Oncorhynchus nerka*) under constant and cyclic temperatures in relation to live zooplankton ration size. Can. J. Fish. Aquat. Sci. 37: 203–210.

Growth rates of Babine Lake underyearling sockeye salmon (*Oncorhynchus nerka*) fed rations of zooplankton of 14–84 cal/kcal sockeye·d^{-1} (1 cal = 4.1868 J; 1.3–7.8% of dry body wt) were determined at constant temperatures of 6.2, 11.3, 15.3, and 15.9°C, and under a cyclic temperature regime similar to that encountered during their diel vertical migrations. Growth of fish fed rations of 43–76 cal/kcal·d^{-1} (4.0–6.9% of dry body wt) was equal or greater under cyclic temperatures than constant temperatures. At the lowest and highest rations growth was greater, respectively, under the constant low temperature and the constant high temperature than under cyclic temperatures. Gross growth efficiency, ranging from <1 to 31%, was affected by temperature and ration in much the same way as growth. Young sockeye ingesting moderate rations comparable to those probably available in the relatively warm surface layers of Babine Lake, and undergoing diel migrations from deep cold waters to the surface and return, are likely to grow more rapidly and efficiently than fish maintained at constant temperatures. The selective value of vertical migrations in thermally stratified waters may have an energetic basis as suggested by some other workers.

Key words: sockeye salmon, growth rate, conversion efficiency, diel vertical migration, temperature, zooplankton ration, diel cyclic temperature

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Les taux de croissance de saumons nerka (*Oncorhynchus nerka*) de moins d'un an du lac Babine, nourris à des rations de 14–84 cal/kcal par sujet·d^{-1} (1 cal = 4,1868J; 1,3–7,8% du poids corporel sec) ont été déterminés à des températures constantes de 6,2, 11,3, 15,3 et 15,9°C, et à un régime de températures cycliques semblable à celui auxquels ils sont exposés durant leurs migrations verticales nycthémérales. La croissance des poissons nourris à des rations de 43–76 cal/kcal·d^{-1} (4,0–6,9% du poids corporel sec) à des températures cycliques est égale ou supérieure à celle à des températures constantes. Aux rations les plus basses et les plus élevées, la croissance est plus grande, respectivement, à la basse température constante et à la haute température constante qu'aux températures cycliques. L'efficacité de croissance brute, variant de <1 à 31%, est affectée par la température et la ration à peu près de la même façon que la croissance.

Les jeunes saumons nerka absorbant des rations modérées comparables à celles qu'ils trouvent probablement dans les couches superficielles relativement chaudes du lac Babine et effectuant des migrations nycthémérales depuis les eaux froides profondes jusqu'à la surface et retour croîtront probablement plus rapidement et plus efficacement que les poissons maintenus à des températures constantes. Il est possible que la valeur sélective des migrations verticales dans des eaux thermiquement stratifiées ait un fondement énergétique, ainsi que l'ont suggéré certains autres chercheurs.

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DIEL vertical migrations of fish and zooplankton occur commonly and are well documented. Typically such migrations take the animals from colder hypolimnetic waters in late afternoon or early evening to warmer surface waters where feeding occurs. Return to the hypolimnion usually occurs around dawn. An example of this type of behavior has been described by Narver (1970) for underyearling sockeye salmon (*Onco-*

[1]Based in part on a thesis submitted by R. M. Biette in conformity with the requirements for the degree of Doctor of Philosophy, at Simon Fraser University, Burnaby, B.C.

[2]Present address: Fisheries Branch, Ontario Ministry of Natural Resources, Queen's Park, Toronto, Ont. M7A 1W3.

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rhynchus nerka) in Babine Lake, B.C. Fish leave the hypolimnion (5–9°C) at dusk and move to the epilimnion (12–18°C) where feeding occurs over 1–2 h. Following feeding, young sockeye descend to the upper layer of the thermocline (12–15°C) but move back into the epilimnion before dawn, feed briefly and descend to the hypolimnion for the remainder of the day.

The adaptive significance of such diel vertical migrations has been the subject of considerable speculation. Current explanations center on a) a possible reduction in rate of predation on migrants, b) enhanced efficiency of utilization of available or ingested food, or c) some combination of the two.

McLaren (1963) suggested that the reduced metabolic rate of poikilotherms in the cooler, deeper water to which migrants descend when not feeding could leave more of the ingested food energy available for growth and reproduction. Metabolic and growth studies led Brett (1971a) to conclude that McLaren's "energy bonus" hypothesis might account for the diel vertical migrations of underyearling sockeye in Babine Lake. Enright (1977) developed a conceptual model which also indicated a metabolic benefit to diel vertical migration of *Calanus helgolandicus* (*pacificus*) in thermally stratified waters.

Several workers (Lock and McLaren 1970; Kelso 1972; and Swift 1976) have examined McLaren's hypothesis experimentally and reported slower growth in animals exposed to a cyclic temperature regime (as experienced by diel migrants) than in those grown at constant high or intermediate temperatures. They concluded that McLaren's energetic model did not hold.

An alternative explanation of the adaptive significance of diel vertical migrations not involving energetic considerations was proposed by McLaren (1974). He has shown that size and fecundity of *Pseudocalanus minutus* are inverse functions of temperature and suggests that the larger migrants resulting from exposure to low temperatures coupled with a presumed reduction in predation in the hypolimnion could lead to a selective advantage over nonmigrants in surface waters.

The work described here tests the hypothesis that growth of young Babine Lake sockeye salmon fed controlled and limiting rations of live zooplankton is greater in fish maintained under cyclic temperatures (comparable to those experienced during diel vertical migrations) than constant temperatures. We have attempted to duplicate the environmental experience of the young sockeye to the extent possible while still conducting controlled experiments.

Materials and Methods

Experiments were conducted during the summers of 1969 and 1970 at the Smithers Landing field station of Fisheries and Oceans Canada located on the North Arm of Babine Lake, B.C. The 1969 experiments were exploratory and established the relationship of growth to certain fixed temperatures and natural rations. Two major sets of experiments were conducted in 1970 (from June 23–July 25, and July 28–August 29) and are referred to as Expt. 1 and Expt. 2, respectively. A factorial experimental design was utilized in both with three temperatures and five rations as variables.

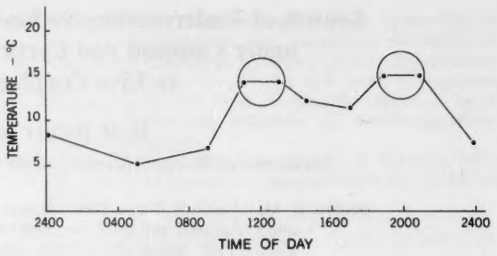

FIG. 1. The cyclic temperature regime to which underyearling sockeye salmon were exposed during Expt. 1 and 2. Feeding occurred during the periods of maximum temperature indicated by the large circles.

Underyearling sockeye salmon used in the experiments were obtained in Babine Lake with a beach seine or purse seine. Fish were transported to the field station, sorted to an average wet weight of 199 ± 60 mg (±2 SE) in Expt. 1 and 966 ± 70 mg (±2 SE) in Expt. 2, randomly distributed to experimental tanks (36 fish per tank), and then held for 2–4 d under the temperature regime to which they were subsequently exposed during the experiments. Fish were held in 15 197-L oval dark green fiberglass tanks housed in a shed with a white translucent roof and open sides. Each tank was fitted with a dark lid covering the entire tank. A continuous supply of lake water was pumped from depths of 1 and 12 m and filtered through 80-μm nylon netting into two large storage tanks which provided approximately 2 L of water per minute to each experimental tank. Water from 12 m was refrigerated to achieve hypolimnetic temperatures.

The three temperatures used in Expt. 1 were 15.3 ± 1.8°C, 11.3 ± 1.0°C (±2 SD), and a cyclic temperature regime which ranged from 4.5 to 17.5°C (Fig. 1) and approximated that experienced by young sockeye in the course of their diel vertical migrations in Babine Lake. In Expt. 2 temperatures were 15.9 ± 1.7°C, 6.2 ± 0.4°C (±2 SD), and the same cyclic regime indicated in Fig. 1. Expt. 1 and 2 were designed to examine the effect of an intermediate (~10°C) and low (~5°C) temperature, respectively. The temperatures actually achieved (11.3 and 6.2°C, respectively) reflect the capabilities of our water pumping and mixing systems. Cyclic and intermediate temperatures were produced by altering the percentage of epilimnial and hypolimnial water dispensed into the tanks.

Live zooplankton used as food was collected daily from Babine Lake surface waters using 253 or 308-μm mesh size Nitex plankton nets. The samples were strained gently through a series of sieves to obtain animals of fairly uniform size (<1.5 mm; predominantly *Diaptomus ashlandi*, *Cyclops scutifer*, and *Bosmina coregoni*). Species composition and moisture content of the food were determined at regular intervals.

Fish were provided one-half their daily ration at 11:00 and 19:00. The feeding times coincided with the maximum

water temperatures of the daily cyclic regime (Fig. 1). Tank covers were removed and water pumps stopped during the 2-h feeding interval. The daily rations, which were obtained by drawing off different volumes of zooplankton from well-stirred net samples, were 1.3, 4.0, 5.4, and 6.7% and 1.4, 4.1, 5.5, and 6.9% of fish dry body weight in Expt. 1 and 2, respectively. In addition, some tanks were provided with a quantity of food in excess of that which the fish could consume during the 2-h feeding period. Residual food in these tanks was collected on three occasions each week, dried, and weighed. The average of these values was assumed to be representative of other days and was used in calculating food ingestion when excess food was provided. The absolute quantity of food provided each day was adjusted at 10-d intervals to compensate for measured changes in fish biomass.

Logistic reasons necessitated feeding fish at the times noted above. Thus, fish were fed at higher light intensities and different times of day than those in nature. We do not believe this has affected our conclusions. Since all food offered was consumed (except in the surplus ration experiments) any impact of our experimental regime could only be on incorporation efficiency or fate of incorporated energy. Biette (1978) did not observe any differences in the fate of ^{14}C-labeled food in fish held under cyclic temperatures and fed at 11:00 or 19:00.

All fish were weighed and fork length measured at the beginning of experiments and at 10-d intervals thereafter. Fish were lightly anesthetized with 2-phenoxyethanol (0.26 mL/L) and blotted on paper towels to remove surface moisture before weighing and length measurement. Fish were not fed on weighing days in order to eliminate variability in weight introduced by food in the gut. After 33 d (including weighing days) all fish were sacrificed, samples taken for dry weight determinations, and the remainder quick frozen and stored for later caloric measurements. Dry weight of fish and rations were measured after oven drying at 60°C for 48 h.

Instantaneous growth rate (g) was calculated using the least-squares method. Specific growth rate ($G = g \times 100$) was also determined. Growth rate was also expressed in terms of changes in ash-free caloric content determined on representative dried fish and zooplankton samples with a Phillipson micro-bomb calorimeter standardized using benzonic acid (6318 cal/g). Ash content was determined by oxidizing samples of fish and zooplankton in a muffle furnace at 600°C. Ash values were subtracted from dry weights to calculate ash-free caloric values.

Gross growth efficiencies were determined by caloric comparison of the quantity of fish tissue elaborated and food ingested. Gross growth efficiency ($\Delta B/C \times 100$) is the ratio of growth to total food ingested, where ΔB is average growth rate in cal/kcal of fish·d^{-1} and C is ration. Maintenance rations (R_{maint}), defined as the point at which the smoothed growth-ration curve intersected the zero growth line, were determined for each temperature regime (Fig. 3).

Results

Measurement of growth rates showed that noticeable ration and temperature dependent size differences were apparent within the first 10 d of both experiments and were generally maintained throughout the 33-d study duration (Fig. 2). Specific growth rates associated with

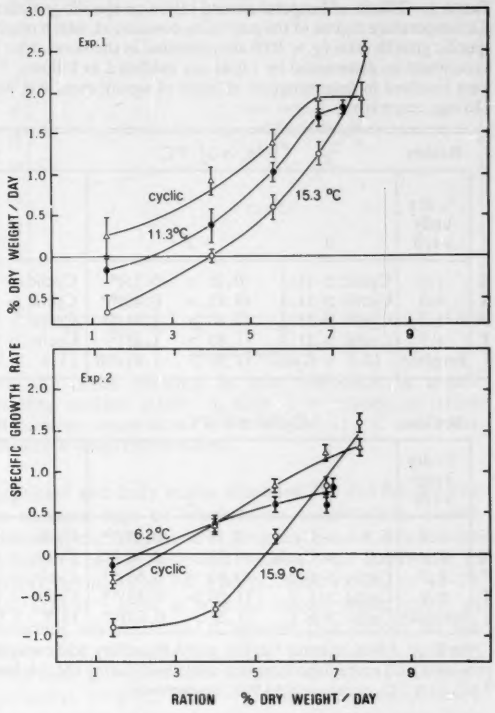

FIG. 2. Relation of specific growth rate to ration for underyearling sockeye salmon held at constant temperatures of 15.3°C (○), 11.3°C (●), and the diel cyclic temperatures (△) in Expt. 1; and 15.9°C (○), 6.2°C (●), and diel temperatures (△) in Expt. 2. The cyclic temperature regime is depicted in Fig. 1. Limits are $\bar{x} \pm 2$ SE.

the various temperature and ration regimes were generally significantly different (Table 1) although there were instances of nonsignificance. Specific growth rates in Expt. 1 were in the following order in all cases *except where surplus food was provided*: cyclic temperature regime > 11.3°C > 15.3°C (Table 1). At the lowest ration (1.3% of dry body weight/d) positive growth was recorded only by fish grown under the cyclic temperature regime (Fig. 2). Lowest and highest growth rates were observed in fish grown at 15.3°C with rations of 1.3 and 7.8%/d, respectively (Fig. 2).

The Expt. 2 growth rates exhibited the same general trends though were lower than those observed in Expt. 1, an expected result given the differences in initial fish size and the lower temperatures in one of the constant temperature experiments. At lowest ration (1.4% of dry body wt/d) growth rates were: 6.2°C > cyclic temperature regime > 15.9°C (Fig. 2, Table 1). At rations of 5.5 or 6.9% of dry body wt/d growth was

TABLE 1. Effects of temperature and ration on specific growth rates of Babine Lake underyearling sockeye salmon. In Column 1 the temperature regime of the pair being considered, which resulted in most rapid fish growth is presented first. The corresponding specific growth rates ($g \times 100$) are presented in the same order in Column 2. Comparisons of specific growth rates over the 33-d experiment as determined by *t*-tests are indicated as follows: $^{**}P < 0.01$; $^{*}P < 0.05$; NS = not significant. In excess of 60 df were involved in determination of levels of significance. Wet weights of fish at the beginning of Expt. 1 and 2 averaged 199 and 966 mg, respectively.

	Ration	Cyclic vs 11.3°C		Cyclic vs 15.3°C		11.3°C vs 15.3°C	
	% dry body wt/d	1	2	1	2	1	2
E	1.3	Cyclic > 11.3	(0.25 > −0.15)*	Cyclic > 15.3	(0.25 > −0.70)**	11.3 > 15.3	(−0.15 > −0.70)**
X	4.0	Cyclic > 11.3	(0.87 > 0.40)**	Cyclic > 15.3	(0.87 > 0.04)**	11.3 > 15.3	(0.40 > 0.04)**
P	5.4	Cyclic > 11.3	(1.43 > 1.01)**	Cyclic > 15.3	(1.43 > 0.57)**	11.3 > 15.3	(1.01 > 0.57)**
T.	6.7	Cyclic > 11.3	(1.83 > 1.58)*	Cyclic > 15.3	(1.83 > 1.40)**	11.3 > 15.3	(1.58 > 1.40)NS
1	Surplus[a]	11.3 > Cyclic	(1.99 > 1.81)NS	15.3 > Cyclic	(2.50 > 1.81)**	15.3 > 11.3	(2.50 > 1.99)**

	Ration	Cyclic vs 6.2°C		Cyclic vs 15.9°C		6.2°C vs 15.9°C	
	% dry body wt/d	1	2	1	2	1	2
E	1.4	6.2 > Cyclic	(−0.17 > −0.37)**	Cyclic > 15.9	(−0.37 > −0.89)**	6.2 > 15.9	(−0.17 > −0.89)**
X	4.1	6.2 > Cyclic	(0.33 > 0.30)NS	Cyclic > 15.9	(0.30 > −0.77)**	6.2 > 15.9	(0.33 > −0.77)**
P	5.5	Cyclic > 6.2	(0.83 > 0.61)**	Cyclic > 15.9	(0.83 > 0.23)**	6.2 > 15.9	(0.61 > 0.23)**
T.	6.9	Cyclic > 6.2	(1.20 > 0.58)**	Cyclic > 15.9	(1.20 > 0.88)**	15.9 > 6.2	(0.88 > 0.58)**
2	Surplus[a]	Cyclic > 6.2	(1.23 > 0.80)**	15.9 > Cyclic	(1.62 > 1.23)**	15.9 > 6.2	(1.62 > 0.80)**

[a]In Expt. 1 fish ingested 7.2, 7.7, and 7.8% of dry body weight/d at 11.3°C, cyclic temperature, and 15.3°C, respectively, when provided with more food than was consumed during the 2-h feeding periods. Corresponding values in Expt. 2 were 7.1, 7.6, and 7.6% at 6.2°C, cyclic, and 15.9°C, respectively.

greatest under cyclic temperatures while growth rates in fish provided with surplus food were: 15.9°C > cyclic temperatures > 6.2°C (Fig. 2, Table 1). Minimum and maximum growth rates were recorded in fish grown at 15.9°C and provided with the lowest and highest rations, respectively (Fig. 2).

The mean caloric values of fish tissues were 6.02 and 6.14 kcal/g (1 cal = 4.1868 J) ash free dry wt. in Expt. 1 and 2, respectively. Ration and temperature regimes did not produce significant differences in caloric content. Average caloric values of the zooplankton fed to the fish were 6.33 and 6.79 kcal/g ash free dry weight in Expt. 1 and 2, respectively.

The relationships of average relative growth rates (cal increment/kcal of fish·d^{-1}) are shown in Fig. 3. The trends observed were similar to those calculated from weight changes (Fig. 2). Maintenance rations (R_{maint}) were also derived from Fig. 3. In only one case was it necessary to extrapolate linearly to the zero growth line (Expt. 1, cyclic temperature). The R_{maint} values expressed as cal/kcal fish·d^{-1} for each temperature regime are as follows:

Experiment 1		Experiment 2	
Cyclic regime	6	Cyclic regime	34
11.3°C	20	6.2°C	25
15.3°C	42	15.9°C	58

The gross growth efficiencies calculated from Expt. 1 data are presented in Fig. 4. Minimum (0.43%) and maximum (31%) values were observed at 15.3°C at rations of 43 and 82 cal/kcal fish·d^{-1}, respectively. Gross growth efficiency was affected by both temperature and ration. Increased rations were generally associated with increased conversion efficiencies although a decline was noted in fish grown under cyclic temperatures and provided with surplus food (Fig. 4).

Similar results were obtained in Expt. 2 (Fig. 5). At a ration of 46 cal/kcal fish·d^{-1} gross growth efficiency under cyclic temperatures did not differ significantly from that in fish grown at constant low temperature. At rations of 61 and 76 cal/kcal fish·d^{-1} the gross efficiency was greatest under cyclic temperature. When surplus food was provided gross conversion efficiencies were highest (20%) at 15.9°C (Fig. 5). Temperature influenced gross conversion efficiencies and growth rates in much the same way.

Discussion

This study has examined the hypothesis that the diel vertical migrations exhibited by many animal species in thermally stratified waters results in more rapid growth than shown by nonmigrants which experience relatively constant temperatures. Our work in-

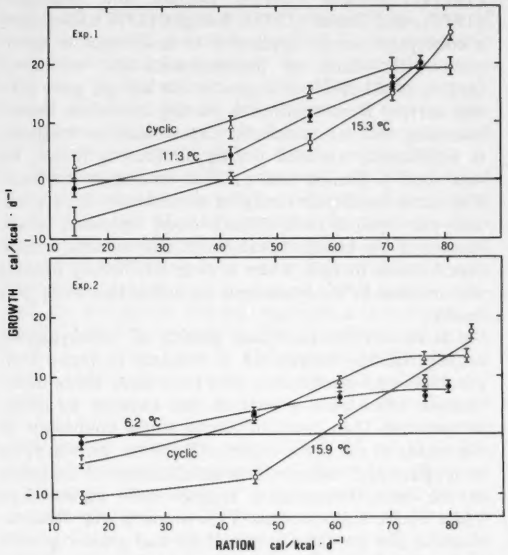

FIG. 3. Relation of average relative growth rate in calories (1 cal = 4.1868 J) to ration for underyearling sockeye salmon held in Expt. 1 at constant temperatures of 15.3°C (○), 11.3°C (●), and under cyclic temperatures (△) and at 15.9°C (○), 6.2°C (●), and cyclic temperatures (△) in Expt. 2. The cyclic temperature regime is depicted in Fig. 1. Limits are $\bar{x} \pm 2$ SE.

volved comparison of growth rates of underyearling sockeye salmon (*Oncorhynchus nerka*) fed known rations and held under temperature regimes which simulated those experienced by vertical migrants and nonmigrants. If fish grown under cyclic temperatures (migrants) grew more rapidly than those grown at constant temperatures (nonmigrants), then the selective advantage of this behavior might reside, at least in part, in the presumed improved rate of survival of these larger fish. Data suggesting that larger or more rapidly growing fish have better survival are generally scarce. However, Foerster (1954) has shown that the percentage return of adult sockeye increases as the size of smolts migrating seaward increases, and Robinson and Barraclough (1978) have shown a 2.5 times increase in the lake survival of the more rapidly growing young sockeye, resulting from the fertilization of Great Central Lake, B.C.

The data we obtained provide considerable, though qualified, support for the hypothesis that growth of young sockeye is greater under cyclic than constant temperatures. Thus, those fish whose daily ration was 4.0–6.9% of dry body weight/d grew as rapidly, and usually more rapidly, when maintained under cyclic temperatures than constant temperatures of 15.9, 15.3, 11.3, or 6.2°C (Fig. 2). Only when surplus food was

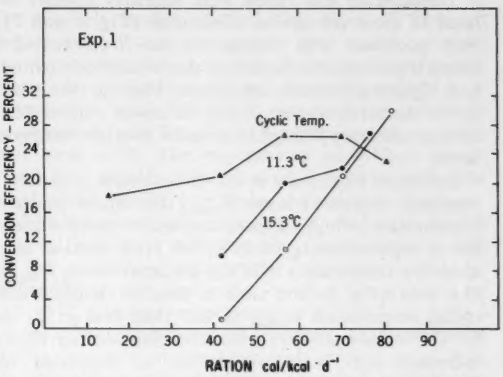

FIG. 4. Gross efficiency of food conversion in underyearling sockeye salmon in Expt. 1 in relation to ration at constant temperatures of 15.3°C and 11.3°C and under the cyclic temperature regime.

provided and daily intake exceeded 7% did fish growth at constant high or intermediate temperatures exceed that at cyclic temperatures (Fig. 2). Similar conclusions emerge when growth and rations were expressed in caloric terms (Fig. 3). Fish provided rations of 43–76 cal/kcal·d^{-1} grew at least as rapidly under cyclic as constant temperatures. It appears that rations do not have to be reduced much below surplus levels to result in greater growth of fish maintained under cyclic temperatures (Fig. 2, Expt. 1). The cyclic temperature enhancement of growth relative to that at constant high or intermediate temperatures persists at all rations below surplus. A constant low temperature (6.2°C) only results in greater growth (or a smaller weight loss in some cases) than in fish exposed to cyclic temperatures when rations are low.

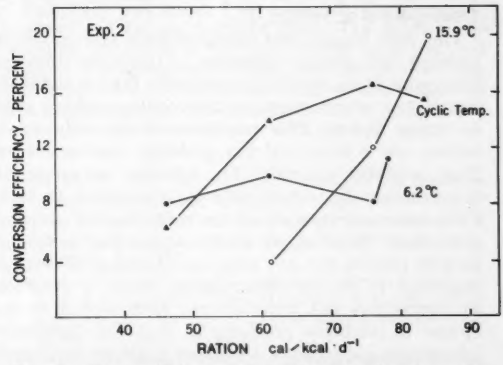

FIG. 5. Gross efficiency of food conversion in underyearling sockeye salmon in Expt. 2 in relation to ration at constant temperatures 15.9°C and 6.2°C and under the cyclic temperature regime.

Temperature and ration were generally directly related to gross conversion efficiencies (Fig. 4 and 5). Fish provided with rations of 45–70 cal/kcal·d^{-1} which is perhaps comparable to that available in nature, had highest conversion efficiencies when grown under cyclic temperatures. At higher or lower rations conversion efficiency was greatest under constant temperatures.

Enhanced efficiencies in fish grown under cyclic temperatures implies a) lower R_{maint} than under constant intermediate or high temperatures and b) more efficient use of ingested energy than in fish maintained at constant low temperature with the presumed lower R_{maint}. Our data (Fig. 3, text table in Results) indicate that cyclic temperature R_{maint} is less than that at 11 or 15°C. We have no data which indicate whether R_{maint} increases with increasing ration as suggested by Paloheimo and Dickie (1966b). If such changes do occur, they are apparently in the same direction for fish in all temperature regimes, since the differences between cyclic and the higher temperatures are consistent in all but those fish provided with surplus food. Growth under cyclic temperatures equalled or exceeded that at constant low temperatures at all rations except the lowest (1.3% of body weight/d). Since the 6.2°C R_{maint} is lower than the cyclic temperature value, it can only be assumed that the higher growth under cyclic temperatures reflects a change in R_{maint} with ration size or a reduction in the amount of energy excreted.

Growth rates and conversion efficiencies measured in this study were amongst the lower published values. Growth rates, which were directly related to ration size (except at surplus food levels), were <2.5%/d. This, and the relatively low gross conversion efficiencies (<25%), probably reflects the zooplankton diet provided to these fish. Yearling sockeye fed marine zooplankton grew more slowly than we observed (Brett 1971b) while sockeye fry provided a commercial granulated food achieved growth rates of 6.5%/d (Shelbourn et al. 1973).

Our data suggest that young sockeye salmon which undergo diel vertical migrations in thermally stratified lakes grow more rapidly and efficiently than would their nonmigrant counterparts residing continuously in cold or warm waters. *This conclusion holds only when rations are submaximal* but probably comparable to those available naturally. The selective advantage of this behavior in sockeye salmon is assumed to stem from increased survival of the more rapidly growing individuals. However, we do not suggest that metabolic benefits provide the only selective advantage of vertical migration in this or other species. There is evidence to suggest that this behavior may have evolved in response to predation pressures or that both metabolic advantages and predator avoidance might be implicated as suggested by Enright and Honegger (1977).

Our results, with their bioenergetic basis, are in general agreement with those of McLaren (1963), Brett (1971a), Enright (1977), Enright and Honegger (1977), and Clarke (1978). Enright (1977) developed a conceptual model applicable to herbivores or predators with which he demonstrated that nocturnal feeding could result in a greater net energy gain than that derived from continuous feeding in surface waters assuming that a) metabolic expenditure of migrants is significantly reduced during nonfeeding hours; b) that food is limited and c) that feeding is noctural. The latter assumption may be unnecessary in a predator–prey system such as ours. Food limitation was a feature of our experimental design and reduced energy expenditures in cold water during nonfeeding periods was implicit in the hypothesis on which this study was based.

Clarke (1978) examined growth of underyearling sockeye salmon maintained at constant or cyclic temperatures and provided surplus food three times daily. Growth rates were greater in fish exposed to cyclic temperature than constant temperatures equivalent to the means of the cyclic regimes. However, growth rates at constant high temperatures which exceeded the mean of the cyclic temperature regimes were higher than under cyclic temperatures. This supports our observation that fish provided surplus food had greater growth under high than cyclic temperatures.

Hokanson et al. (1977) reported more rapid growth in rainbow trout fed to satiation 5 times/d under cyclic than constant temperatures providing they were below the 17°C optimum. At higher temperatures most rapid growth occurred under constant temperatures. Our results generally support those of Hokanson et al. though they noted a benefit from cyclic temperatures at the lower temperatures (<17°C) even when surplus food was provided.

While some studies suggest growth enhancement on exposure to cyclic temperatures, this has not invariably been the case. Lock and McLaren (1970) and Swift (1976) noted more rapid growth rates in animals maintained at constant high temperatures than cyclic temperatures. This may be attributable to ration size. Lock and McLaren provided surplus food to the *Psuedocalanus minutus* used in their experiments. There is some indication that ration was not limiting in Swift's studies since the *Chaoborus trivittatus* fourth instar larvae grew at the same rate when fed the high and low rations. It may be that any energetic advantage resulting from exposure to cyclic temperatures also disappears in these species when they are provided with excess food.

Kelso (1972) noted that conversion efficiencies in age II–VI walleye, *Stizostedion vitreum vitreum*, grown at constant 12°C, 16°C, and 20°C were consistently higher than in fish held under an 8–16°C cyclic temperature regime. Species, size, or development stage differences may account for the differences between sockeye and walleye. The work of Spieler et al. (1977) may also offer a possible explanation. They reported a temporal interaction of daily thermoperiod

with daily photoperiod which influenced the rate of body and gonad growth in goldfish. Greatest increase was noted when food, which was in excess, was provided in the last 4 h of darkness. Laboratory experiments in which fish are fed at times of day or temperatures not comparable to those in nature may yield results which are difficult to interpret ecologically.

McDonald (1973) has examined the diel vertical movements and feeding habits of underyearling sockeye in Babine Lake. His data provided the basis for comparison of growth rates and conversion efficiencies we measured with those in a natural population. Young sockeye in Babine Lake in August, 1967 had an average dry weight of 336 mg, ingested a dry weight of 14 mg/d (4.2% of body weight) and increased in dry body weight by 2.9%/d (McDonald 1973). Ration and weight increases were converted to cal/kcal·d^{-1} using the caloric values we determined (6.08 kcal/g for fish and 6.56 kcal/g for zooplankton). On this basis, the daily growth and ration were 29 and 45 cal/kcal·d^{-1}, respectively and gross growth efficiency was 64%. At a ration of 45 cal/kcal·d^{-1} the growth rates in our experiments under cyclic temperatures were 10 and 3 cal/kcal·d^{-1} in Expt. 1 and 2, respectively (Fig. 3) which corresponds to gross growth efficiencies of 22 and 7% (Fig. 4 and 5). The 3–9-fold difference between field and laboratory results could be attributed to a) an underestimate of the ration in nature; b) an overestimate of growth rates of wild fish or c) laboratory experiments which do not simulate natural conditions.

Although there is little information on rates of food intake in nature, it seems unlikely that McDonald (1973) has seriously underestimated the ration. Fish in our experiments provided with unlimited food for 2-h periods twice a day consumed maximum daily rations of 7.1–7.8% of dry body weight (78–84 cal/kcal·d^{-1}). Young sockeye provided unlimited quantities of commercial fish food three times daily ingested 10–14% of their dry body weight (Brett et al. 1969). A daily ration in nature of 45 cal/kcal (4.2% of dry body weight) seems reasonable. The high rations provided experimentally are not likely available in nature.

Differences in observed growth rates of wild and experimental fish may result from an overestimate of growth in natural populations. Parker (1971) pointed out that growth of wild *O. gorbuscha* and *O. keta* could be overestimated 0.3–0.5% of body length/d because of predator selection of smaller individuals. McDonald (1973) calculated growth in length for *O. nerka* in Babine Lake from July 5 to August 24 as approximately 0.9% of length/d. As much as 50% of the growth calculated for wild fish may be due to the selective predation effect described by Parker.

There is also evidence that tank studies do not provide accurate estimates of growth in nature (Paloheimo and Dickie 1966a; Kerr 1971). Tank experiments likely underestimate the energetic costs in nature of prey search and vertical migration although both may be relatively modest. On the other hand, we have not estimated the impact on growth rates of the sustained swimming required of sockeye during our experiments. Increased metabolic costs and decreased growth compared with wild fish which are probably relatively quiescent in cold hypolimnetic waters for much of the day seem likely. Our experiments have likely underestimated growth rates in natural populations while McDonald (1973) may have overestimated them.

The estimated daily ration of wild Babine Lake fish (45 cal/kcal·d^{-1}) is within the range where growth in fish maintained under cyclic temperatures was significantly greater than under constant temperatures of 11–15°C (summer temperatures of Babine Lake epilimnion). Our results suggest therefore, that growth of young sockeye undertaking diel vertical migrations in this lake would be greater than for nonmigrants remaining in warmer surface waters.

While growth under cyclic temperatures and at 6.2°C did not differ appreciably (Fig. 2), little of ecological significance can be attached to this similarity. Fish living continuously in the hypolimnion would be unlikely to obtain the rations provided in tank studies or would spend considerable energy collecting the sparsely distributed zooplankton. Food conversion in nature is probably less efficient under constant low temperatures than indicated from laboratory studies.

Thus, at rations comparable to those probably available in the surface waters of Babine Lake the hypothesis that the diel vertical movements of young sockeye results in the highest growth and the most efficient conversion of the available food is supported. This study provides data which suggest that the selective advantage of vertical migrations in thermally stratified waters may reside in the more efficient incorporation of food energy.

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Correlation and Prediction of Metal Toxicity to Aquatic Biota

KLAUS L. E. KAISER

Department of the Environment, National Water Research Institute, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6

KAISER, K. L. E. 1980. Correlation and prediction of metal toxicity to aquatic biota. Can. J. Fish. Aquat. Sci. 37: 211–218.

Published data on the toxicity of metal ions to aquatic biota, in particular *Daphnia magna*, were analyzed for their correlation with ion specific physico-chemical parameters. Significant correlations were obtained for three groups of ions with similar electron configurations in the outer orbitals. Group I ions include Na (I), Be (II), Ba (II), Al (III), and Cr (VI) with inert gas-like electron configurations; group II ions include Cr (III), Mn (II), Fe (III), Co (II), Ni (II), Cu (II), Zn (II), As (V), Cd (II), Pt (IV), Au (III), and Hg (II) with partially or completely filled *d* electron orbitals; group III ions include Sn (II), As (III), Se (IV), and Pb (II), with filled *d* and *s*, but unfilled *p* electron orbitals. The toxicity of an ion within a group can be calculated from the general equation

$$pT = a_0 + a_1 \log \frac{AN}{\Delta IP} + a_2 \Delta E_0$$

where pT is the negative logarithm of a metal ion concentration with a certain toxicity in $mol \cdot L^{-1}$; AN is the metal's atomic number; ΔIP is the difference between the ion's ionization potential with the oxidation number (OX) and the ionization potential of the next lower oxidation number ($OX-1$) in electron volts (eV); and ΔE_0 is the absolute value of the electrochemical potential between the ion and the first stable reduced state. The values for the constants a_0, a_1, and a_2 depend on the group of ions, the biota, and the particular toxic effect determined. The equation is applied to predict the toxicities of other ions under similar test conditions.

Key words: toxicity, metals, ions, correlations, *Daphnia magna*, aquatic tests

KAISER, K. L. E. 1980. Correlation and prediction of metal toxicity to aquatic biota. Can. J. Fish. Aquat. Sci. 37: 211–218.

Nous avons analysé les données publiées sur la toxicité d'ions métalliques dans les biocénoses aquatiques, en particulier *Daphnia magna*, afin de déterminer s'il y a corrélation entre ceux-ci et des paramètres physico-chimiques spécifiques aux ions. On a trouvé des corrélations significatives chez trois groupes d'ions à configurations électroniques identiques dans les orbitales externes. Les ions du groupe I comprennent : Na (I), Be (II), Ba (II), Al (III) et Cr (VI) à configurations électroniques semblables à celles des gas inertes; les ions du groupe II comprennent : Cr (III), Mn (II), Fe (III), Co (II), Ni (II), Cu (II), Zn (II), As (V), Cd (II), Pt (IV), Au (III) et Hg (II) à orbitales électroniques *d* partiellement ou entièrement remplies; les ions du groupe III comprennent : Sn (II), As (III), Se (IV) et Pb (II) à orbitales électroniques *d* et *s* remplies, mais *p* non remplie. L'équation générale suivante peut servir à calculer la toxicité d'un ion au sein d'un groupe :

$$pT = a_0 + a_1 \log \frac{AN}{\Delta IP} + a_2 \Delta E_0$$

où pT est le logarithme négatif d'une concentration d'ions métalliques d'une toxicité donnée en $mol \cdot L^{-1}$; AN est le nombre atomique du métal; ΔIP est la différence entre le potentiel d'ionisation de l'ion dont le nombre d'oxydation est OX et le potentiel d'ionisation du nombre d'oxydation immédiatement au-dessous ($OX - 1$), en électron volt (eV); et ΔE_0 est la valeur absolue du potentiel électrochimique entre l'ion et le premier état réduit stable. Les valeurs des constantes a_0, a_1 et a_2 dépendent du groupe d'ions, de la biocénose et de l'effet toxique particulier trouvé. A l'aide de cette équation, nous prédisons la toxicité d'autres ions dans des conditions d'essais identiques.

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TOXICITY threshold values can be considered as the activities, or, as an approximation, the concentrations of metal ions at their upper limits of physiological compatibility. Irrespective of an element's[1] function in

[1]The term "element" here refers to an element's spectrum of ionic and covalently bound derivatives.

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a biological system, such as plant or animal, it will impart a toxic effect to that system when present at a concentration above its toxicity threshold. Such toxicity thresholds vary widely among the elements. Furthermore, the sequence of toxicity thresholds of a series of ions may vary dramatically between different organisms.

Mendeleev, in his "system of periods,"[2] recognized fundamental relations of the elements which made it possible to predict correctly some of the physical and chemical properties of previously undiscovered elements. However, any biological properties, such as toxicity threshold values of metal ions, have proven difficult to correlate and predict. One problem here has been the extreme variation of threshold values found among ions of chemically similar elements. For example, the toxicities of magnesium and aluminum can differ by several orders of magnitude. Among the possible reasons for such differences are factors derived from the size and charge of the metal ions as well as the type and abundance of the corresponding ligands present (Tevlin 1968). Particularly strong variations of toxicity are also found between related transition metals, for example, iron and cobalt. Moreover, it has been shown that the toxicity of many elements also varies strongly with the oxidation state.

In contrast with our present inability to predict metal toxicity, the correlation and prediction of biological effects of organic compounds has proven quite successful. Based on pioneering research, notably by the schools of C. Hansch and T. Fujita, quantitative structure-activity correlations (QSAR) of organic compounds are now an important tool for the development of new pharmaceutical drugs. In fact, both the search for new, biologically active, basic drug structures, and the optimization of the desired medicinal effects draw heavily on QSAR applications.

This paper describes correlations of ion specific, physical parameters with previously reported toxicity thresholds of a comparatively simple organism, the aquatic invertebrate *Daphnia magna*. Based on these correlations, the toxicity thresholds of other ions of unknown toxicity are predicted.

Results

The present study examined a variety of multiple linear correlations between some physico-chemical parameters of the elements and their concentrations causing a chronic toxicity (3 wk, 16% reproductive impairment) to *Daphnia magna*, as reported by Biesinger and Christensen (1972). By trial and error, it was found that there are certain element characteristics which can be used to calculate toxicity values which compare well with those experimentally observed. In addition, the found correlations may be applied to predict the toxicities of other ions. The calculations also showed it to be necessary to separate the ions into groups with distinct electronic parameters. These groups and parameters were found to be: (i) ions with completely filled *p* orbitals, such as Al (III), Cr (VI), and Ba (II); (ii) ions with partly or completely filled *d* orbitals, such as Fe (III), Co (II), As (V), Ag (I), and Hg (II); and (iii) ions with filled *s* orbitals, such as As (III), Sn (II), and Pb (II).

[2]The "Table of periods" is commonly known as the "Periodic table" of the elements, however, there is little *periodicity* in this table and it would be better referred to as a table of periods, or better yet, a "table of element groups and periods."

Background

In an in-depth study, Biesinger and Christensen (1972) determined the toxicity thresholds of 21 metals and metalloids to *Daphnia magna* in Lake Superior water of a mean hardness 45.3 $mg \cdot L^{-1}$. They found the toxicity thresholds to vary from 0.68 $g \cdot L^{-1}$ for sodium, the least toxic, to 0.17×10^{-6} $g \cdot L^{-1}$ for cadmium, the most toxic of the elements investigated. The complete list of values observed by Biesinger and Christensen (1972) and their transformation into logarithmic numbers is given in Table 1; where $pTm = -\log (mol \cdot L^{-1})$. Biesinger and Christensen (1972) tried to correlate the observed thresholds with some known physical and chemical parameters of the elements, but with limited success. Linear correlation coefficients were determined between the concentrations (−log molarity) of selected metal ions causing a 3-wk, 16% reproductive impairment to *Daphnia magna* and the solubility product of the metal sulfides ($r = 0.78$; $n = 9$), the electronegativities ($r = 0.74$, $n = 20$), and the equilibrium constants of the metal-ATP complexes ($r = 0.74$, $n = 11$). They further concluded that no definite correlations for this effect were obtained with such physical parameters as standard electrode potential, covalent radius, ionic radius, first ionization energy, electrical conductivity, atomic weight, atomic number, density, and equilibrium constants of certain metals with a variety of organic ligands, including cysteine, aspartic acid, histidine, arginine, glycylglycine, glutathione, and carboxypeptidase A.

Alkali and Alkaline Earth Metals

The observed toxicities (pTm) of alkali, alkaline earth metal, and aluminum ions, as reported by Biesinger and Christensen (1972), were found to correlate well with the values predicted by equation (1), with standard deviations in parentheses:

$$(1)\quad pTm = 11.63(\pm 0.09) + 4.87(\pm 0.17) \log \frac{AN}{\Delta IP} - 4.29(\pm 0.14)\Delta E_0$$

where AN is the atomic number of the element, ΔIP the difference of the ionization potential in volts between its oxidation number (OX) and the next lower one ($OX - 1$), and ΔE_0 the absolute value of the electrochemical potential in volts between the ion with the

TABLE 1. Concentrations of ions effecting in 3-wk a 16% reproductive impairment of *Daphnia magna* in Lake Superior water. Data from Biesinger and Christensen (1972).

Metal	*OX*[a]	$\mu g \cdot L^{-1}$	$\mu mol \cdot L^{-1}$	*pTm*[b]
Na	I	680 000	29 600	1.53
Ca	II	116 000	2 890	2.54
Mg	II	82 000	3 400	2.47
K	I	53 000	1 400	2.85
Sr	II	42 000	480	3.32
Ba	II	5 800	40	4.40
Fe	III	4 380	78	4.11
Mn	II	4 100	75	4.12
As	V	520	6.9	5.16
Sn	II	350	2.9	5.54
Cr	III	330	6.3	5.20
Al	III	320	12.0	4.92
Zn	II	70	1.1	5.96
Au	III	60	0.3	6.52
Ni	II	30	0.51	6.29
Pb	II	30	0.14	6.85
Cu	II	22	0.34	6.47
Pt	IV	14	0.072	7.14
Co	II	10	0.16	6.80
Hg	II	3.4	0.017	7.77
Cd	II	0.17	0.0015	8.82

[a]*OX* = oxidation number.
[b]$pTm = -\log (mol \cdot L^{-1})$.

oxidation number *OX* and its first stable, reduced form, the elemental state of these metals. Equation (1) fits the data for Na, K, Mg, Ca, Sr, Ba, and Al with a highly significant correlation coefficient $r = 0.981$ ($n = 7$). The correlation coefficient increases to $r = 0.9985$ ($n = 6$) if the value for potassium is excluded. The observed toxicity of K (I) is greater than the calculated value. This might easily occur if the metal salt had trace impurities with high toxicity. This minor discrepancy possibly suggests a redetermination of the observed toxicity of potassium. The observed toxicity values (*pTm*) and those calculated from the regression line, excluding the experimental value for potassium, are given in Table 2. Also shown in Table 2 are the calculated toxicity values (*pTc*) for a variety of other elements in oxidation states for which the equation (1) is predicted to apply and it should be emphasized here that equation (1) is only valid for the particular oxidation numbers of the elements as given in Table 2.

The range of experimental toxicity values (*pTm*) in this group (Table 2) comprises approximately 3.5 orders of magnitude, with sodium (I) being the least and aluminum (III) the most toxic of these ions. The calculated toxicity values (*pTc*) for all ions in Table 2 span almost 8 orders of magnitude with sodium (I) being the least and manganese (VII) the most toxic ion in this group.

TRANSITION METAL AND RELATED IONS

The toxicity values (*pTm*) of the transition metal

TABLE 2. Electrochemical potentials (ΔE_0), ionization potential differentials (ΔIP), observed (*pTm*) and calculated (*pTc*) toxicities of alkali, alkaline earth, and other metals; calculated from equation (1) for elements with the oxidation numbers (*OX*) given.

Element	*OX*	ΔE_0[a] (volts)	ΔIP[a] (electron volts)	*pTm*[b]	*pTc*
Na	I	2.71	5.14	1.53	1.61
K	I	2.92	4.34	2.85	2.22
Rb	I	2.93	4.18	—	3.69
Cs	I	2.92	3.89	—	4.70
Be	II	1.70	8.89	—	2.65
Mg	II	2.38	7.39	2.47	2.45
Ca	II	2.76	5.76	2.54	2.42
Sr	II	2.89	5.34	3.32	3.38
Ba	II	2.90	4.79	4.40	4.39
Al	III	1.71	9.62	4.92	4.93
Si	IV	1.73	11.64	—	4.60
Sc	III	2.08	11.95	—	3.90
Y	III	2.37	8.27	—	4.74
La	III	2.37	7.74	—	5.69
Ac	III	~2.6[c]	7.9	—	5.60
Ti	IV	0.86[d]	15.77	—	8.64
Zr	IV	2.32	9.88[c]	—	4.63
Ce	IV	1.45	13.3	—	8.53
V	V	1.00[d]	17.2[c]	—	7.95
Cr	VI	1.10[d]	17.4[c]	—	7.59
Mn	VII	0.59	21[c]	—	9.47

[a]Values from Chemical Rubber Company (1978).
[b]Values from Biesinger and Christensen (1972), see Table 1, this paper.
[c]Values from Cotton and Wilkinson (1967).
[d]Values for acidic conditions.

(groups III B, IV B, V B, VI B, VII B, and VIII) ions with oxidation numbers (*OX*) less than their group numbers, and metal ions of the groups I B and II B and those of the elements of the fourth to seventh main groups with oxidation numbers equal to their group numbers, were found to correlate well with the toxicities calculated by equation (2), with standard deviations in parentheses:

$$(2)\quad pTm = 4.80(\pm 0.32) + 6.15(\pm 0.45) \log \frac{AN}{\Delta IP} - 3.11(\pm 0.32)\Delta E_0$$

where *pTm*, *AN*, and ΔIP are defined as for equation (1) and where ΔE_0 is the absolute differential in volts of the standard potential of the ion with the oxidation number *OX* and the ion in the next lower oxidation state which is stable under the test conditions. This linear regression, calculated by the least-squares method from the toxicities of 12 ions measured by Biesinger and Christensen (1972) is also found to be significant at the 99.9% confidence limit with a correlation coefficient $r = 0.978$ ($n = 12$). Table 3 lists such ions in this category, the toxicity values (*pTm*) observed by Biesinger and Christensen (1972) and the values calculated (*pTc*) from the equation (2) for the same

TABLE 3. Electrochemical potentials (ΔE_0), ionization potential differentials (ΔIP), observed (pTm), and calculated toxicities (pTc) for transition metals and main group metalloids; calculated values from equation (2) for elements with the oxidation numbers (OX) given.

Element	OX	ΔE_0[a] (volts)	ΔIP[a] (electron volts)	pTm[c]	pTc
Cr	III	0.41	14.46	5.20	4.88
Mn	II	1.03	8.21	4.12	4.57
Mn	IV	1.21	18.31	—	1.87
Fe	II	0.41	8.28[b]	—	6.58
Fe	III	0.77	14.46	4.11	3.97
Co	II	0.28	9.19	6.80	6.81
Ni	II	0.23	10.52	6.29	6.70
Cu	II	0.16	12.57	6.47	6.53
Zn	II	0.76	8.57	5.96	5.78
Ga	III	0.56	10.13	—	6.04
Ge	IV	0.12	10.49	—	7.40
As	V	0.71	12.5	5.16	5.19
Se	VI	0.03	14.0	—	7.07
Br	VII	0.61	14.4	—	5.27
Pd	II	0.83	11.09	—	6.02
Ag	I	0.80	7.57	—	7.19
Cd	II	0.40	7.91	8.82	8.37
In	III	0.34	9.17	—	8.22
Sn	IV	0.15	10.23	—	8.57
Sb	V	0.59	11.9	—	6.85
Pt	II	1.2	9.60[b]	—	6.66
Pt	IV	0.74	12.6	7.14	7.37
Au	I	1.68	9.22	—	5.31
Au	III	1.29	10.4	6.52	6.20
Hg	II	0.91	8.32	7.77	8.01
Tl	III	0.37	9.38	—	9.40
Pb	IV	0.28	10.38	—	9.45

[a]Values from Chemical Rubber Company (1978).
[b]Values from Cotton and Wilkinson (1967).
[c]Values from Biesinger and Christensen (1972), see Table 1, this paper.

and additional ions. The range of observed toxicities in this group comprises approximately 4.5 orders of magnitude with iron (III) and cadmium (II) as the two extremes. In contrast, the calculated toxicities of all ions in this group ranges from a low of $pTc = 1.87$ for manganese (IV) to a high of $pTc = 9.45$ for lead (IV), comprising more than 7.5 orders of magnitude.

Discussion

It is apparent that both equations (1) and (2) follow the general equation (3):

$$(3)\quad pTm = a_0 + a_1 \cdot \log \frac{AN}{\Delta IP} + a_2 \cdot \Delta E_0$$

with different constants a_0, a_1, and a_2 according to the group of ions in question. Of particular interest here is the ratio of the constants a_1 and a_2 for the two equations (1) and (2). For equation (1) the ratio of $a_1/a_2 = 1.14$ is nearly half that of those in equation (2), where $a_1/a_2 = 1.98$. This may be interpreted that, for a given value $AN/\Delta IP$ the toxicity of ions following equation (1) are more sensitive to changes of ΔE_0 than for those ions following equation (2). The reasons for this can be seen in the electronic structure of the respective ions. For those ions for which equation (1) applies, their oxidation states are equal to their group numbers; therefore, such ions have electron configurations with completely filled s and p orbitals identical to the inert gases preceding their position in the table as, for example, Cr (VI), and Ar (0). In contrast, the ions following equation (2) have outer electron configurations of partly or completely filled d electron orbitals. In general, such d orbitals are more easily subject to hybridization and distortion through the influence from surrounding ligands including solvating water molecules. In comparison to the alkali metals, this higher dependence of ΔE_0 of the transition metals and related ions on the surrounding ligands may be the reason for the large differences between their respective toxicities, such as, for example, between Fe (III) and Co (II).

Other Ions of Groups III A to V A

Two of the ions for which toxicity values were measured by Biesinger and Christensen (1972), namely Sn (II) and Pb (II), were found not to follow either equation (1) or (2). This does not appear surprising in view of the above interpretation. In contrast to the ions listed in Tables 2 and 3, Sn (II) and Pb (II) have only completely filled s and d orbitals. It can be expected, therefore, that such ions will follow the general equation (3) with a different set of constants a_0, a_1, and a_2 than in the equations (1) and (2). Other ions which should follow equation (3) are Tl (I), Sb (III) and Bi (III).

Biesinger and Christensen (1972) have measured only two ions of this group. To be able to estimate the constants for equation (3), we need a set of at least three toxicity values. Anderson (1948) measured threshold concentrations of metal salts producing immobilization of *Daphnia magna* in Lake Erie water and among the toxicity values determined were those for Sn (II), Sb (III), and Pb (II). As his value for Sn (II) is quite uncertain, only the Sb and Pb values are useful for calculations here. Because of the different conditions and effects observed from those of Biesinger and Christensen (1972), it is further necessary to apply a correction factor to Anderson's data to make them comparable to the data of the latter. This is achieved by adding a constant value of $\Delta pTm = -1.10$ to the values found by Anderson (1948), as determined by the difference in the toxicity of Pb (II) between that value and that of Biesinger and Christensen (1972). The same correction factor applied to Sb (III) gives an estimated toxicity value of $pTm = 5.29$ for comparison with the values found by Biesinger and Christensen (1972). We now have the required minimum of three toxicity data to determine

TABLE 4. Electrochemical potentials (ΔE_0), ionization potential differentials (ΔIP), observed (pTm), and calculated toxicities (pTc) of metals and metalloids; values calculated from equation (4) for elements with the oxidation numbers (OX) given.

Element	OX	ΔE_0[a] (volts)	ΔIP[a] (electron volts)	pTm[b]	pTc
As	III	0.68	9.71	—	3.75
Se	IV	0.35	11.0	—	3.32
Sn	II	0.14	7.29	5.54	5.54
Sb	III	0.66	8.8	5.29[c]	5.29
Tl	I	0.34	6.11	—	7.55
Pb	II	0.13	7.61	6.85	6.85
Bi	III	0.46	8.88	—	6.59
Hg	I	0.80	10.43	—	6.17[d]

[a]Values from Chemical Rubber Company (1978).
[b]Values from Biesinger and Christensen (1972).
[c]Values estimated from Anderson (1948), see text.
[d]See text.

the corresponding constants for equation (3). The resultant equation (4) should be valid for ions with filled d and s electron orbitals as indicated before:

$$(4)\quad pTm = -0.13 + 6.70 \log \frac{AN}{\Delta IP} + 0.46\,\Delta E_0$$

where pTm, AN, ΔIP and ΔE_0 are defined as previously. Table 4 lists toxicity values calculated from equation (4). Of course, it should be kept in mind that the constants in equation (4) were derived from the minimum of observed values and are therefore less reliable than those computed for the equations (1) and (2). Consequently, the calculated toxicities of these ions (Table 4) are subject to a larger error. Particularly uncertain is the value for Hg (I), as there is no comparison with any other ion with a half filled s orbital. However, if the general relationship of equation (2) holds true, namely that ions both with partially and completely filled d orbitals follow the same formula, it follows that ions with partially filled s orbitals, such as Hg (I), may also follow equation (4).

TOXICITY AND OXIDATION STATE

It is now of interest to examine the toxicity values of elements occurring in different oxidation states. A number of such cases can be found in the Tables 2, 3, and 4 and are summarized in Table 5. For example, the metals Cr, Mn, Fe, Pt, As, Au, Tl, Pb, and Sn, commonly occur in more than one oxidation number. Examination of the data in Table 5 shows that for all ion pairs, except for Fe (II)/Fe (III) and Mn (II)/Mn (IV), the ions with the higher oxidation numbers have the higher calculated toxicity. This effect of the oxidation number on the metal's toxicity is particularly strong for the elements Cr, Mn, Sn, and Se, where the corresponding toxicity values differ by three or more

TABLE 5. Calculated toxicity values pTc[a] for elements commonly found in two or more oxidation states.

Element	OX[b] I	OX II	OX III	OX IV	OX V	OX VI	OX VII
Cr			4.88			7.59	
Mn		4.57		1.87			9.47
Fe		6.58	3.97				
As			3.75		5.19		
Sn		5.54		8.57			
Sb			5.29		6.85		
Pt		6.66		7.37			
Au	5.31		6.20				
Hg	6.17	8.01					
Tl	7.55		9.40				
Pb		6.85		9.45			
Se				3.32		7.07	

[a]Calculated toxicity values from Tables 2, 3, and 4.
[b]Oxidation number.

orders of magnitude. This observation coincides with the fact that their higher oxidation states (e.g. Cr (VI)) are strong oxidants. In contrast, Fe (II) and Mn (II), particularly in a basic medium, are reducing agents.

The calculated toxicity values in Table 5 indicate that elements in oxidation states either above or below those normally found in oxygenated natural waters, such as Fe (II), Cr (VI), Mn (II), or Mn (VII), will be more toxic to biota than when in their more common oxidation states, i.e. Fe (III), Cr (III), or Mn (IV). This is in agreement with experimental observations on the toxicities of $MnCl_2$ ($pTm = 3.40$) and $KMnO_4$ ($pTm = 5.40$) to *Daphnia magna* by Anderson (1944 and 1948). The calculated toxicity values for As (III) and As (V), however disagree with the generally higher toxicity of As (III). (Sorensen 1976; Gilderhus 1966).

It is known that the presence of complexing ligands, whether inorganic or organic, such as phosphate ions, humic acid, etc. can effect a change in the standard potentials and therefore, also of ΔE_0 of the central metal ions. Ideally, for the toxicity calculations according to equation (3), the ΔE_0 values should be determined in natural waters after equilibration of the ions with the ligands present. Such determinations, of course, would also account for effects on the ΔE_0 due to the pH, temperature, ionic strength, and other parameters for the particular water in question. In general, no such measurements can be made because of the lack of sensitivity of analytical instruments at the low enivronmental concentrations of importance. The quality of fit of the regression equations (1) and (2), as determined from the values of the correlation coefficients, is thought to reflect the high water quality and low hardness of the Lake Superior water. Stronger changes of the actual ΔE_0 values, hence also of the toxicities, are to be expected for waters with higher concentrations of natural and introduced organic and inorganic compounds which will alter the complexing

TABLE 6. Correlation coefficients (r), number of ions (n), and constants a_0, a_1, and a_2, for toxicity correlations per equation (3) for various aquatic biota and enzymes.

Species or enzyme system	Exposure time	Effect	Ions	n	r	a_0	a_1	a_2	Reference
American oyster, *Crassostrea virginica*	12 d	LC05	Ni, Cu, Ag, Hg	4	0.900[a]	4.61	6.99	−4.11	Calabrese et al. (1977)
Mummichog, *Fundulus* sp.	168 h	LC50	Ni, Zn, Cd, Hg	4	0.961[b]	4.15	3.68	1.17	Eisler and Hennekey (1977)
Rainbow trout (*Salmo gairdneri*)	96 h	TL50	Cr (III), Ni, Cu, Zn, Ag, Cd	6	0.865[b]	2.10	6.62	−0.89	Hale (1977)
Daphnia hyalina	48 h	LC50	Cs, Mg, Sr, Cr (VI)	4	0.994[c]	9.25	3.19	−3.00	Baudouin and Scoppa (1974)
Daphnia hyalina	48 h	LC50	Co, Ni, Hg, Zn, Cd, Cu	6	0.573	4.47	1.88	1.02	Baudouin and Scoppa (1974)
Eudiaptomus padonus	48 h	LC50	Co, Ni, Hg, Zn, Cd, Cu	6	0.648	4.08	1.03	0.41	Baudouin and Scoppa (1974)
Cyclops abyssorum	48 h	LC50	Co, Ni, Hg, Zn, Cd, Cu	6	0.749[a]	3.18	1.67	0.03	Baudouin and Scoppa (1974)
Fish carbonic anhydrase	—	I50	Mn (II), Fe (III), Co, Ni, Cu, Zn, Pd (II), Ag, Cd, Pt (IV), Au (III), Hg (II), Tl (III), Sn (IV)	14	0.60[a]	1.46	2.18	0.92	Christensen and Tucker (1976)
Fish carbonic anhydrase	—	I50	Na, Be, Ca, Al, Cr (VI)	5	0.944[b]	5.15	2.70	−1.73	Christensen and Tucker (1976)
Glutamic oxalacetic transaminase	—	I20	Fe (III), Co, Ni, Cu, Zn, Pd (II), Pt (IV), Ag, Hg (II), Cd, Ge (IV)	11	0.782[b]	6.70	2.64	0.90	Christensen (1971/72)
Lactic dehydrogenase	—	I20	Mn (II), Fe (III), Co, Cu (I), Cu (II), Zn, Cd, Hg (II), Pd (II), Au (III), Ge (IV), Ag	12	0.686[b]	7.03	2.34	0.42	Christensen (1971/72)

Statistical significance: [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.

capacity and therefore the electrochemical potentials of the metal ions. (Chau et al. 1974).

APPLICATION TO OTHER ORGANISMS

The correlations of experimentally observed toxicity data, such as in the equations (1) to (4) are of importance when trying to predict the effect of other ions to the same organisms under the same conditions. However, the usefulness of such correlations would be considerably enhanced if any of the equations (1), (2), or (4) could also be applied to predict the effects of these ions on other biota and, possibly even to enzyme systems directly. Attempts were made to see whether other biological responses, besides the 3-wk 16% reproductive impairment of *Daphnia magna*, can be correlated with elemental parameters by applying equation (3).

The literature abounds with reports on various toxic effects of metal ions to a great number of aquatic species. However, studies of one particular species and a particular chronic effect caused by many different agents, such as the one by Biesinger and Christensen (1972), are less common. Table 6 gives the results from a few such studies. Correlation coefficients r, and constants a_0, a_1, and a_2 were obtained from calculating linear best-fit curves for the observed toxic effects of metal ions to a variety of aquatic species. Metal ion concentrations were transformed to logarithmic values and the ions were grouped with an electron configuration as in the noble gases (e.g. Na, Be, Ca, Al, Cr (VI)), and into groups with partially filled d orbitals (e.g. Ni, Cu, Ag, Hg), as described before.

Baudouin and Scoppa (1974) measured the acute toxicity (48-h LC-50) of various metal ions to three species of invertebrates, including *Daphnia hyalina*. Their selection of metals also includes Cs and Cr (VI), not investigated by Biesinger and Christensen (1972). For the reasons given previously, Cs (I) and Cr (VI) are expected to follow the relationship of equation (1). The correlation of the toxicity values observed by Baudouin and Scoppa (1974) of four ions in this series (exclusive of their value for calcium) gives a correlation factor of $r = 0.994$ ($n = 4$) with the values for constants a_0 to a_2 as given in Table 6. Calculations of the toxicities (pTc) from this equation give the following results, with the experimentally observed data (pTm) in parentheses: Cs (I) 4.25 (4.31), Mg (II) 2.97 (2.88), Ca (II) 2.69 (1.13), Sr (II) 2.94 (3.07), Cr (VI) 6.34 (6.37). Examination of these values in relation to the sublethal effect values (pTc) listed in Table 2, shows that both series can be interrelated by equation 5:

$$(5)\quad pTc_{D.\ hyalina} = -1.19 + 1.38\, pTc_{D.\ Magna}$$

where $pTc_{D.h.}$ and $pTc_{D.m.}$ are the calculated ion concentrations for the 48-h LC50 for *Daphnia hyalina* and the 3-wk 16% reproductive impairment for *Daphnia magna*, respectively. Equation (5), of course, is applicable only to those ions also following equation (1); its correlation coefficient is $r = 0.987$ ($n = 5$).

Other short- to medium-term toxicity series of ions include those of Ni, Zn, Cd, and Hg to the mummichog (*Fundulus heteroclitus*) (Eisler and Henneky 1977), and those of Ni, Cu, Ag, and Hg to the American oyster (*Crassostrea virginica*) (Calabrese et al. 1977). Hale (1977) determined the acute toxicity of several transition metal ions to rainbow trout (*Salmo gairdneri*). The least-squares best-fit curve of these data results in the constants as given in Table 6. The data correlate reasonably well ($r = 0.864$, $n = 6$) with the following calculated values (pTc) for the toxicities (experimental values in parentheses): Cr (III) 3.19 (3.33), Ni (II) 4.71 (3.22), Cu (II) 4.36 (5.40), Zn (II) 5.02 (5.08), Cd (II) 6.92 (7.23), Ag (I) 6.63 (6.57). Except for Ni and Cu, good agreement of the observed and calculated values is obtained.

Baudouin and Scoppa (1974) also investigated the acute toxicities of Co, Ni, Zn, Cu, Cd, and Hg to three species of invertebrates. The correlation factors (Table 6) determined for these are lower than for the previous

ion group but it is thought that the applicability of toxicity correlations to short-term tests is generally less than those to long-term studies with smaller toxicant concentrations. Therefore, for the purposes of toxicity correlations, studies of chronic effects are to be preferred.

An interesting series of data is available on the 50% inhibition of the in vitro activity of the channel catfish (*Ictalurus punctatus*) enzyme carbonic anhydrase by a large number of metal ions (Christensen and Tucker 1976). Table 6 gives the constants and correlation coefficients for ions grouped as for the equations (1) and (2). Good agreement is obtained for the alkali group ions, including Be (II) and Cr (VI). The correlation of 14 ions, including Pd, Ag, Pt (IV), Au (III), and Tl (III) appears less satisfying with a correlation coefficient of $r = 0.60$ ($n = 14$). Similar studies were made by Christensen (1971/72) on the effects of metal salts on the in vitro activities of the enzymes glutamic oxalacetic transaminase and lactic dehydrogenase. Table 6 shows the resulting constants and correlation coefficients and their statistical significance. There is little improvement over the correlations calculated by Christensen (1971/72) with several physical parameters such as the electronegativity of the elements and various reaction constants with common anions, such as sulfide and ATP. It is not immediately clear why these poor correlations are found; however, an examination of the concentration/effect profiles obtained by Christensen (1971/72) and Christensen and Tucker (1976) shows a great variability of the slopes as well as nonlinearity of several of the concentration/effect curves. For example, to produce the range of 0–100% enzyme inhibition, the concentration of Na (1) ranges over nearly five orders of magnitude while the same range of inhibition is observed with a Hg (II) concentration varying only one order of magnitude. As a result, it becomes quite speculative at which effect level, if any, a useful correlation may be attempted. Additional complications arise because of the fact that some concentration/effect profiles show nonlinearity with one or more inflections.

Theoretical Considerations

Attempts to correlate the biological activities of ions with some of their physico-chemical constants are not new. Matthews (1904) demonstrated a significant relationship of metal ion toxicity to eggs of *Fundulus* sp. with the standard electrode potential. Shaw (1954) proved general correlations of toxicity using a term *pI* comprising the susceptibility to and the toxicities of metal ions to several species of aquatic biota. Metal ion toxicity was found to be affected by the amount of available or free ions as determined from the solubility products of the respective metal sulfides. (Shaw (1961) expanded his theory to propose the general toxicity scale of Mn (II) < Fe (II) < Co (II) < Ni (II) < Cu (II) > Zn (II) based on the principles of coordination chemistry. Indeed, he demonstrated that this sequence held true for most experimental observations with *Daphnia magna*, threespine stickleback (*Gasterosteus aculeatus*) guppy (*Lebistes reticulatus*), tadpoles (*Bufo valliceps*), and also the enzymes urease and diastase. The above toxicity sequence conforms with the Irving and Williams (1953) order on the sequence of complex stabilities for the above metals with such ligands as EDTA, glycinate and oxalate. Both the molar toxicities and the complex stabilities are highest for the Cu (II) ion.

Christensen and Tucker (1976) determined the concentrations of metal ions producing a 50% inhibition of the in vitro activity of the enzyme carbonic anhydrase. Among the metals tested, Hg (II) had the strongest and Co (II) the least effect. In terms of their respective molar concentrations, their activity differed by approximately four orders of magnitude. This is in contrast to several in vitro studies where the toxicity of Co (II) was about equal to that of Hg (II), such as to *Daphnia* sp. (Biesinger and Christensen 1972; Baudouin and Scoppa 1974). There is, of course, no reason to expect a similar toxicity sequence when comparing metal effects on one particular enzyme with those on a living organism where each of many enzymes may be acted upon differently by a given metal ion. Furthermore, transport mechanisms through the body membranes will be controlling at least some aspects of an ion's toxicity.

Life depends on the concurrent function of all enzyme and membrane systems in the body. Such systems, however, show different individual sensitivities to a given set of toxicants. For example, an enzyme very sensitive to sodium ions is likely to be different from one very sensitive to mercury ions, and even the order of increasing toxicity of a series of metal ions may not be the same for both enzymes. Usually, however, several enzymes control an integrated body function, such as reproduction. Therefore, a gross physiological effect, such as reproductive impairment is the result of the body's integrated response to the superimposed, individual toxicant effects on each enzyme. As it appears reasonable to presume that different biological species of similar complexity, such as fishes, have similar sets of enzymes, it may be postulated that such species also show similar *sequences* of sensitivity to a given set of metal ions. Indeed, there is experimental evidence, such as referred to previously, to support that view. Moreover, such assumptions were widely used to develop environmental standards and objectives with the help of application factors (for example Water Quality Board 1975).

The interpretation of complex formation and stability of metal ions with inorganic and organic ligands has greatly been improved by the introduction of the concept of "hard" and "soft" ions, as pioneered by Edwards (1954) and Schwarzenbach (1961). This concept has been expanded by Pearson (1963) and by Klopman (1968). The ion specific "softness," *En*, cal-

culated by Klopman (1968) from orbital and desolvation energies can be used to predict and explain the stability of metal-ligand complexes and the reaction rates of organic molecules.

The empirical equation (3), as reported herein, contains two variables ΔIP and ΔE_0, which are measures for ion specific energies. The term ΔIP is similar to the orbital energy calculated by Klopman (1968). The standard potential difference ΔE_0 can be viewed as a measure of an ion's ability, under actual conditions in aqueous solutions, to change its electronic state. This ability is certainly related to the desolvation energy calculated by Klopman (1968) but does include other solvent and ligand controlled effects on the ions. In a simplified view, equation (3) can be interpreted as showing the toxicity of an ion as a function of its atomic ionisation potentials (ΔIP) and its observed electronic behavior (ΔE_0) in the solvent/ligand system in question. The third, element specific component in equation (3), the atomic number (AN) of the element, may be interpreted as a measure of the ion's molecular size or inertia. Its combination with the other parameters allows the successful correlation of elements having similar ΔIP and ΔE_0 values but significantly different atomic sizes, such as for example, sodium and strontium. The correlations reported herein deal with metal ions of the second to the sixth period in the Table of Periods. At present, no useful toxicity data are available to determine their applicability to other elements, such as the lanthanides and actinides. It may be expected, however, that the toxicities of such elements with incompletely filled d orbitals can also be calculated from the equation (3) with a yet unknown set of the constants a_0, a_1, and a_2.

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Role of a Unionid Clam Population in the Calcium Budget of a Small Arctic Lake

ROGER H. GREEN

Department of Zoology, University of Western Ontario, London, Ont. N6A 5B7

GREEN, R. H. 1980. Role of a unionid clam population in the calcium budget of a small arctic lake. Can. J. Fish. Aquat. Sci. 37: 219–224.

Growth rate and morphometric parameters were estimated for the *Anodonta grandis* (Pelecypoda, Unionidae) population in Shell Lake, N.W.T. (68°N), based on sampling during the summer of 1972. Variation in density and size of *Anodonta* within the lake as a function of varying depth and slope of bottom was estimated by SCUBA during the summer of 1973. Predictive models based on these data estimate total calcium in shells of the *Anodonta* population to be 10.1 Mg, which is approximately half the amount of calcium dissolved in Shell Lake water. The youngest four or five year-classes are missing from the estimated age distribution, and recent pollution may be a factor. Independent estimates of calcium used for shell growth, and of calcium released by death of individuals and subsequent shell dissolution, approximate 50% (of the calcium in the *Anodonta* population) per year. This implies a mean residence time in the *Anodonta* population of approximately 2 yr.

Key words: calcium, clam, unionid, lake, arctic

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Un échantillonnage effectué à l'été 1972 a servi à estimer le taux de croissance et les paramètres morphométriques de la population d'*Anodonta grandis* (Pelecypoda, Unionidae) du lac Shell, T.N.-O. (68°N). Des observations en plongée autonome pendant l'été 1973 ont permis d'estimer la variation de densité et de taille des *Anodonta* dans le lac en fonction de la profondeur et de la pente du fond. Des modèles prévisionnels fondés sur ces données estiment que le calcium total des coquilles de la population d'*Anodonta* est de 10,1 Mg, soit approximativement la moitié du calcium dissous dans l'eau du lac Shell. Les quatre ou cinq plus jeunes classes annuelles sont absentes de la distribution estimée des âges, et il est possible qu'une pollution récente soit un facteur. Des estimations indépendantes du calcium utilisé dans la croissance des coquilles et du calcium remis en liberté par mortalités individuelles et dissolution subséquente des coquilles, donnent environ 50% (du calcium présent dans la population d'*Anodonta*) par année. Cela signifie que la durée moyenne de résidence dans la population d'*Anodonta* serait d'approximativement 2 ans.

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Introduction

THE purpose of this study is to assess the role of a large population of the unionid bivalve mollusk *Anodonta grandis* in the calcium budget of a small arctic lake (68° 19'N) bordering on the Mackenzie River Delta. Shell Lake is 5 km southeast of Inuvik, N.W.T., and since 1968 has been used as a commercial float plane base in summer and a ski plane base in winter. It has a surface area of 84.3 ha, a volume of 2060×10^3 m^3, and a maximum depth of 5.6 m (Chang 1975) (Fig. 1). The drainage area is small (ca. 600 ha) and both inflow (east end) and outflow (west end) are intermittent. Rooted macrophytic vegetation is common to a depth of approximately 1.5 m, especially on the relatively flat shallow areas at the east and west ends of the lake. At depths below 2 m the bottom substrate is a flocculent mud (after Folk 1968). There is some evidence of pollution by hydrocarbon fuels, especially in the vicinity of aircraft docking facilities along the north shore; iridescence is often visible both on the water surface and in the substrate surface. Citing personal communications, Chang (1975) suggests that no major oil spills have occurred, but that there are high concentrations of oleoclasic microorganisms and hydrocarbons in the nearshore sediments. Oxygen levels appear to be high at all times, and pH is in the range 6.3–7.2. The calcium concentration during the ice-free season is 0.25 mol·m^{-3} which, given the volume of the lake, represents a standing crop of 20.6 Mg of dissolved calcium in Shell Lake. During winter the calcium concentration in the water increases as the salts are frozen out of the surface ice, doubling to a concentration of 0.50 mol·m^{-3} by May when the ice is thickest (ca. 1.5 m). Ice 1.5 m thick occupies approximately 50% of the lake volume, which agrees with the seasonal

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change in calcium concentration. For additional information on water chemistry of Shell Lake see Campbell et al. (1975).

During a collecting trip through the northern Yukon Territory and the Mackenzie River Valley in the summer of 1972, a large population of *Anodonta grandis* was observed in Shell Lake. Individuals covering as wide a range of sizes as possible were collected and relationships among parameters such as volume, length, shell and tissue weights, and age were evaluated. The age–length relationship was estimated from annual growth rings by the Walford plot technique, as described by Green (1973).

The *A. grandis* population in nearby Boot Lake is characterized by much larger sizes, heavier shells, and faster growth rates. Boot Lake is connected to the Mackenzie River east channel and has a concentration of dissolved calcium ranging from 1.0 to 1.4 $mol \cdot m^{-3}$, which is 3–4 times that of Shell Lake. The *A. grandis* shell weight versus age relationships in the two lakes are contrasted in Fig. 2. Analyses of covariance indicate that both the length–age and the shell weight–length relationships differ between the two lakes ($P < 0.01$).

These results obtained during the summer of 1972 suggested that calcium availability may be a limiting factor to shell growth in lakes such as Shell Lake, and that the large unionid clam population in Shell Lake — with its small drainage area and intermittent inflow–outflow channels — may be an important part of the calcium budget of the lake. The water chemistry of Shell Lake is intermediate between Mackenzie Delta lakes and Precambrian Shield lakes, and thus in a sense is an "average" northern lake. *Anodonta* is one of the most ubiquitous North American unionid genera, found in fresh waters from the Atlantic to the Pacific and from the Caribbean to the Arctic coasts. Unionids are more diverse and abundant in lotic than in lentic environments, but *Anodonta* commonly occurs in lakes and in oligotrophic lakes is often the last unionid to drop out at the low pH, dissolved salts concentration, and productivity end of the range. The species *A. grandis* is distributed throughout the Mississippi–Missouri, Arctic, and Hudson Bay drainages, and is the most common (often the only) unionid species in thousands of arctic and subarctic lakes of the Precambrian Shield. Clarke (1973) discusses possible subspecies of *A. grandis*. For the purposes of this study I assume that *Anodonta* from both lakes are taxonomically the same.

Shell growth rate and adult shell thickness in *A. grandis* (R. H. Green unpublished data) and in other unionid species as well (Green 1972) appear to vary among water bodies as a function of variation in water chemistry, independent of variation in shell shape, tissue growth rate, or other morphological features. Therefore shell size and weight are influenced by water chemistry, and the causal relationship may not be all one-way. At the least the shells of the *A. grandis* population would act as a capacitor in the calcium dynamics

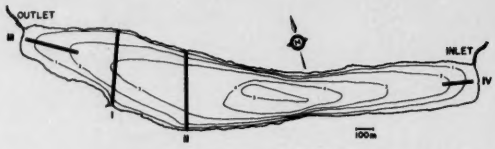

FIG. 1. Bathymetric map of Shell Lake, N.W.T. (after Chang 1975). Four sampling transects are shown.

of the system. For these reasons it seemed worthwhile to study the calcium budget of Shell Lake and the part played by the *A. grandis* population in it. The results may have general implications for management of many low productivity lakes of northern Canada.

Materials and Methods

Sampling was done by SCUBA during August 1973, along four transects which are shown in Fig. 1. Two replicate 2-m^2 samples were collected at depth intervals (Table 1) along each transect. The transect was followed by lining up shoreline features. Depth was determined with a measured line. While I was in the water an assistant in the boat would move along the transect until the next depth was located, and then lower to the bottom a weighted line with an 80-cm line attached to the weight. I then held the line taut and circled the weight, using bare hands to thoroughly search the 2-m^2 circular area defined by the 80-cm line radius. The search for clams in that sample

TABLE 1. Summary data related to density variation of *Anodonta grandis* in Shell Lake.

Transect code	Side of lake	Depth (m)	Slope (m/100 m)	No. of clams in both 2-m^2 replicate samples
I	North	1.0	1.78	0
I	North	1.5	1.56	93
I	North	2.0	1.29	106
I	North	2.5	0.92	28
I	North	3.0	0.25	31
I	Center	3.25	0.21	3
I	South	2.5	1.01	125
I	South	2.0	1.48	145
I	South	1.5	1.85	101
I	South	1.25	2.01	0
II	North	1.25	3.09	0
II	North	2.0	2.70	7
II	North	3.0	2.09	21
II	North	4.0	0.14	16
II	South	4.0	0.14	20
II	South	3.0	2.01	73
II	South	2.0	2.57	0
II	South	1.1	2.98	0
III	West end	1.0	0.51	0
III	West end	1.5	0.46	35
III	West end	2.0	0.41	141
IV	East end	1.0	1.36	0
IV	East end	1.5	1.12	0
IV	East end	2.0	0.85	74

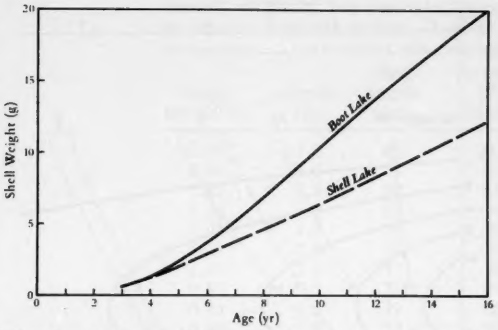

FIG. 2. Estimated shell weight versus age relationships for *Anodonta grandis* in Shell Lake and Boot Lake.

ended when a complete 360° circuit yielded no individuals. Defining a unit effort to be a complete circuit, the catch per unit effort always fell off sharply. Almost all clams were found in the first circuit, or in the first and second if the density was high. Searching was largely by touch because of the easily disturbed flocculent mud. When collection of a particular sample was finished, I would surface and pass the collecting bag to the assistant who would count and measure the lengths of the live clams, record the data, and return the clams to the lake. Then the boat would be moved, either a distance of about 10 m for a second replicate sample or to the next depth. Selection of stations (i.e. given depths on given transects) and of replicate locations at those stations was made entirely by the person in the boat who did not try to and generally could not see the bottom.

A series of specimens covering a wide size range had been collected the previous summer, for determination of relationships among age, length, volume, shell weight, and tissue weight. The Walford plot of the "lengths at consecutive annual rings" data is shown in part A (bottom half) of Fig. 3. The axes extend to sizes of clams that were collected. These go well beyond the data because only consecutive rings that were clear and unambiguous were used, which were generally those representing young ages. A linear relationship $L_{t+1} = a + bL_t$ in the Walford plot implies a Von Bertalanffy growth model $L = L_\infty(1 - e^{-Kt})$ where growth rate $dL/dt = KL_\infty - KL$, decreasing as a linear function of size L. As in some other *A. grandis* populations that I studied, the linear Walford plot model is inadequate. An approximate test of linearity was made by fitting the regression model $L_{t+1} = a + b_1L_t + b_2L_t^2$ and testing for significance of the quadratic term. The null hypothesis that the linear component of the model is an adequate descriptor of the $L_{t+1} = f(L_t)$ relationship is rejected ($P < 0.01$). Although this quadratic model serves as an adequate test of linearity, the general parabolic relationship it describes is not a very satisfying growth model. To derive such a model, I assumed the growth rate to be a declining exponential function of size ($dL/dt = De^{-rL}$) rather than a linear function of size ($dL/dt = KL_\infty - KL$). This results in the growth model $L = (1/r)(\ln[(rD)t + 1])$. In both models the constant of integration is chosen so that size is zero at age zero.

This model may be generally useful beyond the present

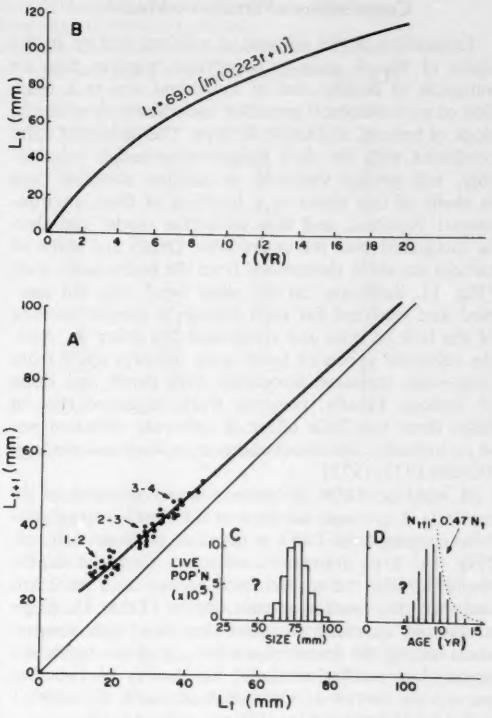

FIG. 3. Part A: Walford plot of lengths at consecutive annual rings for Anodonta grandis in Shell Lake, with probable "length groupings" of the earliest year-classes indicated. The curve through the data is the difference equation form of the model shown in part B. Part B: The estimated length-versus-age relationship. Part C: The estimated size (length in millimetres) distribution for the total population in Shell Lake. Part D: The estimated age distribution, with a descending exponential model fitted to the right-hand limb of the distribution. The question marks in parts C and D indicate uncertainty about the cause(s) of an apparent absence of small, young individuals.

study, and some comments on it are therefore appropriate. First, it is not a generalization of the Von Bertalanffy model. It contains the same number of parameters to be fitted from the data, but assumes a different functional relationship between growth rate and size — one that does not assume an asymptotic size and therefore may be more realistic for many species. Second, it must be fitted iteratively. Third, when $rD = 1$ it is identical to the model proposed by Levinton and Bambach (1970) and shown by them to provide an adequate fit to a wide range of bivalve mollusc growth data. It is possible to make $rD = 1$ for any set of data by scaling units of size and/or time appropriately. However, in this case the fitted values for r and D (0.0145 and 15.3) were used in the model which is shown in parts A and B of Fig. 3.

Construction of Predictive Models

Estimation of the amount of calcium tied up in the shells of the *A. grandis* population requires data on variation of density and of individual size as a function of environmental predictor variables such as depth, slope of bottom, and substrate type. The biological data, combined with the shell weight-versus-length relationship, will predict variation of calcium standing crop in shells of live clams as a function of these environmental variables, and this predictive model can then be integrated over the entire lake. Depth and slope of bottom are easily determined from the bathymetric map (Fig. 1). Substrate, on the other hand, was not sampled and analyzed for each biological sample because of the lack of time and equipment for doing so. Also, the substrate appeared to be quite uniform apart from large-scale variation associated with depth and slope of bottom. Finally, previous work suggested that in lakes there was little effect of substrate variation per se on unionid clam distributions or on their morphology (Green 1971, 1972).

A total of 1019 *A. grandis* were collected in 48 replicate 2-m² area samples at 24 depth-interval stations ranging from 1 to 4 m depth on the four transects (Fig. 1). Live individuals were not found at depths shallower than 1.5 m; densities peaked at 2 or 2.5 m and then decreased at greater depths (Table 1). Slope of bottom appeared to affect densities, with steepest areas having the fewest clams for any given depth. An appropriate predictive model for density N (number per square metre) as a function of depth Z (metres) and slope of bottom S (m/100 m) seemed to be

$$\ln (N + 1) = a + b_1 \ln Z + b_2(\ln Z)^2 + b_3 S.$$

Slope of bottom at each station was estimated from the bathymetric map, using two methods. For each station the shortest distance between the contour lines bracketing the station was converted to slope in m/100 m. Also a polynomial regression was fitted to the depth profile for each transect and then differentiated term by term to yield a predictive model for rate of depth change (i.e. slope of bottom). There was good agreement between the two methods. The fit of these data to the model yielded

$$\ln (N + 1) = 0.558 + 6.89 \ln Z - 4.50(\ln Z)^2 - 0.498S.$$

Each of the three predictor variables accounts for significant variation in the criterion variable, in addition to that accounted for by the other two. Analysis of variance indicates that the full regression model accounts for 68% of the among-station variation in density ($R^2 = 0.68$, $P < 0.01$). It is the among-station variation that is relevant here because the predictor variables have the same values at replicate locations for the same station. The fitted model superimposed on the raw data is shown in Fig. 4.

Variation in the size distribution of individual clams

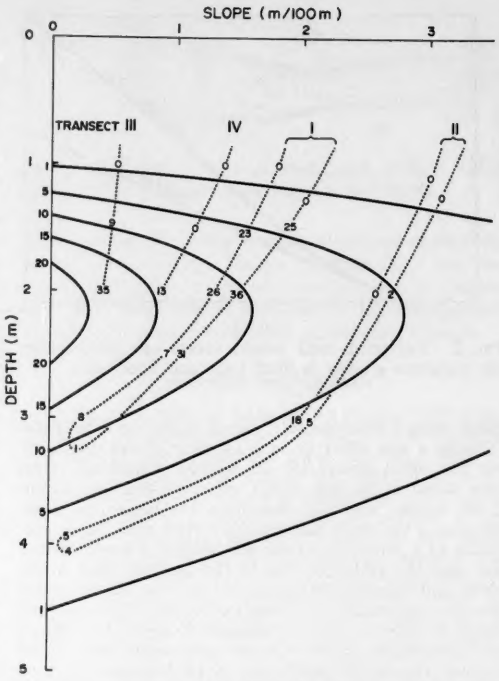

FIG. 4. A representation of the model "$\ln (N + 1) = 0.558 + 6.89 \ln Z - 4.50(\ln Z)^2 - 0.498S$" where N is *Anodonta grandis* density in numbers per square metre, Z is depth in metres, and S is slope of bottom in m/100 m. Isopleths of density are drawn in a two-dimensional depth-versus-slope space, superimposed on the four sampling transects (dotted lines) with the sampling locations and observed mean densities indicated.

as a function of depth and slope of bottom was also considered. Stations yielding fewer than 10 clams in both replicate 2-m² samples were omitted. The data in the right half of Table 2 may be analyzed as a 14-by-3 contingency table, and the resulting χ^2 value indicates that the null hypothesis of similar size-frequency distributions among stations should be rejected ($P < 0.01$). It is apparent that size is related to slope of bottom. The regression of mean length $\bar{L}$ (mm) on depth Z (metres) and slope S (m/100 m) was highly significant, but Z contributes no significant predictive value ($P > 0.05$) about $\bar{L}$ additional to that contained in S. Therefore the regression model used ($r^2 = 0.56$, $P < 0.01$) was

$$\bar{L} = 72.2 + 4.88\,S.$$

The breadth of the size-frequency distribution, as measured by variance in length (see Table 2), also differs among stations (Bartlett's test, $P < 0.01$). The regres-

TABLE 2. Summary data related to size variation of *Anodonta grandis* in Shell Lake. Stations are in order of decreasing slope. Those with fewer than 10 clams are omitted.

Slope (m/100 m)	Depth (m)	Mean length (mm)	Variance of length	No. of clams in both 2-m² replicate samples			
				<70 mm	70–79 mm	>79 mm	Total
2.09	3.0	87	100	2	2	17	21
2.01	3.0	84	85	5	19	49	73
1.85	1.5	80	61	7	40	54	101
1.56	1.5	81	44	1	41	51	93
1.48	2.0	76	56	19	96	30	145
1.29	2.0	74	35	20	72	14	106
1.01	2.5	73	38	36	78	11	125
0.92	2.5	76	79	6	12	10	28
0.85	2.0	79	44	4	35	35	74
0.46	1.5	74	30	8	24	3	35
0.41	2.0	73	26	30	104	7	141
0.25	3.0	72	74	14	12	5	31
0.14	4.0	78	44	2	10	8	20
0.14	4.0	74	86	4	9	3	16
							1009

sion of variance in length, s_L^2, on depth Z and slope S ($R^2 = 0.59, P < 0.01$) is

$$s_L^2 = -16.0 + 21.1Z + 20.5S.$$

Both of the two predictor variables Z and S contribute significantly ($P < 0.05$) to prediction of s_L^2.

Point estimates of density, mean length, and variance in length, based on the above three regression models, were averaged for 173 locations on a 71.5-m grid covering the entire lake. The necessary depth and slope values were estimated from the bathymetric map. From these results, the abundance and the size and age distributions for the entire Shell Lake *Anodonta* population can be calculated (Fig. 3).

The *Anodonta* Population and the Calcium Budget

The size-abundance distribution for the Shell Lake *Anodonta* population was converted to total standing crop of calcium in shells of live clams, in three steps. First, the weight of shell represented by each size class of the population was calculated using the empirical relationship $\ln SW = -8.99 + 2.47 \ln L$ (or $SW = 1.25 \times 10^{-4} L^{2.47}$), where SW is shell weight in grams and L is length in millimetres. Second, it was assumed that all but a negligible portion of the shell weight is $CaCO_3$. This is a reasonable assumption. The organic matrix represents a small proportion of the shell by volume and has a much lower specific gravity than $CaCO_3$. The only other cation likely to be an important constituent of the crystalline shell structure is magnesium, which is present at a concentration half that of calcium in Shell Lake water (Chang 1975). However, aragonite shells tend to be low in magnesium, and unionid shells are typically aragonite (Wilbur 1964). Third, the total weight of *Anodonta* shell — assumed to be $CaCO_3$ — was converted to weight of calcium (using the proportion of 0.400 calcium in $CaCO_3$ by weight) and summed over all size-classes.

The resulting estimate is 10.1 Mg calcium in shells of live *Anodonta* in Shell Lake, which is half of the amount dissolved in Shell Lake water (20.6 Mg — see *Introduction*). If there are no other "compartments" containing large amounts of calcium, then approximately one-third of the calcium in Shell Lake is tied up in the *Anodonta* population. The plankton, and shells left after death of clams, are two possible such compartments. The seasonal cycle of dissolved calcium concentration suggests that plankton are not important in this regard. The calcium concentration drops at the time of ice-out in June and then remains at a stable level — unaffected by plankton blooms — until freeze-up. As for empty clam shells, few were found while sampling for live clams and those which were found tended to be partly dissolved and broken. Since the pH of the water averages less than 7.0, the microenvironment of the substrate surface layer where empty shells occur must be favorable for rapid $CaCO_3$ dissolution — especially during summer.

It is obvious that the estimated size and age distributions include no individuals younger than about 5 yr of age (Fig. 3, parts C and D). There are two possible explanations, either or both of which may be correct. The young individuals may have been there but were not sampled, or they may not have been there. Regarding the former, it is often difficult to find young, small individuals in unionid clam populations — Hunter (1964) specifically mentions *Anodonta* with respect to this problem. They are often at different depths than are the older individuals, and they are easily missed. The sampling method used in this study may have underrepresented small individuals. Screening of sediment samples, which was not done, might have collected younger year-classes. However, the prob-

lem here is not underrepresentation — it is total absence. Of over 1000 individuals sampled the smallest was 57 mm in length. If the youngest three or four year-classes were missing, or very low in density, what might be the explanation? Recruitment may be erratic for natural causes, as it is in many aquatic invertebrate species, and 1973 may have just happened to follow a series of bad years. However, it is interesting that Shell Lake has served as a seaplane base since 1968 and that by 1973, when the work reported by Chang (1975) began, six private bases had been established along the northern side of the lake. By 1973 the resulting hydrocarbon fuel pollution, in both water and sediments, was obvious to Chang and his coworkers and to myself. If recruitment failure caused by pollution is an explanation, then reduction of the ichthyofauna of Shell Lake could be involved. However, *Anodonta* is reported to use a variety of fish species as hosts (Clarke 1973), and local fishermen have continued to catch fish from Shell Lake through the mid-1970s. The most critical stage of the life cycle would probably be when the postglochidial clam breaks free of the host and falls to the bottom in the shallow areas where the fish would tend to feed. These are also the areas receiving most of the oil contamination.

Movement of calcium out of the "*Anodonta* shell" compartment is by death of individual clams and subsequent dissolution of the shells. A descending exponential model fitted to the right-hand limb of the age distribution, as described by Ricker (1958), suggests a mortality rate (and a calcium return rate) of 53% per year (Fig. 3). This estimate is for the oldest part of the population and should be unaffected by the problem of missing early age-classes. Whether this rate would apply to younger age-classes is unknown; mortality rates at intermediate ages might be lower but at the earliest ages would probably be higher. In any case older individuals hold, and would release by shell dissolution after death, a disproportionate amount of the calcium contained in the living population (Fig. 2).

Movement of calcium into this compartment is by uptake for shell growth. Given the age-specific shell growth rates and the observed age distribution (Fig. 3) the annual uptake of calcium for shell growth is estimated to be equal to 40% of the standing crop of calcium in shells of live *Anodonta*. This is probably an underestimate because for younger and smaller clams the absolute shell growth rate, which is fairly constant (Fig. 2), would represent a higher percentage rate. There is good agreement between the two independent estimates, of calcium movement into and out of the clam shell compartment.

A rate of approximately 50% per year implies a residence time in that compartment, for the average calcium atom, of approximately 2 yr. If most of the calcium in Shell Lake is either in dissolved form or in the *Anodonta* population, then a complete cycle — from uptake for shell growth through clam death and shell dissolution to uptake again for shell growth — would average approximately 6 yr.

Acknowledgments

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Mortality, Hematocrit, Osmolality, Electrolyte Regulation, and Fat Depletion of Young-of-the-Year Freshwater Fishes under Simulated Winter Conditions[1]

MICHAEL L. TONEYS[2] AND DANIEL W. COBLE

Wisconsin Cooperative Fishery Research Unit,[3] College of Natural Resources, University of Wisconsin, Stevens Point, WI 54481, USA

TONEYS, M. L., AND D. W. COBLE. 1980. Mortality, hematocrit, osmolality, electrolyte regulation, and fat depletion of young-of-the-year freshwater fishes under simulated winter conditions. Can. J. Fish. Aquat. Sci. 37: 225–232.

In laboratory experiments young-of-the-year green sunfish, *Lepomis cyanellus*, largemouth bass, *Micropterus salmoides*, brook trout, *Salvelinus fontinalis*, and yellow perch, *Perca flavescens*, were held for 4 mo under simulated winter conditions (2–4°C; photoperiod of 9 h light:15 h dark) in solutions of various osmotic strengths, from 90% distilled water to 1.0% NaCl. Mortality in three of five experiments often was greater in salt solutions, indicating that NaCl did not increase survival. Survival was greater for larger yellow perch than smaller but was not related to size in the other species. Hematocrit was depressed below control levels in all solutions at the end of experiments. The fish generally were able to regulate plasma osmotic concentration in all solutions except the highest NaCl concentrations, where plasma osmolality was often significantly higher than in the control at the end of experiments. Plasma Na^+ and Cl^- typically decreased and then recovered at low temperatures. Presence of Na^+ and Cl^- in the external medium tended to alleviate their loss from plasma. Changes in plasma Na^+ and Cl^- were not related to mortality in hypoionic solutions but usually did correspond to changes in plasma osmolality. Fat content of survivors declined and water content increased for all species, but the losses and gains were not related to osmolality of solutions. Low fat content probably was not a cause of death.

Key words: Mortality, electrolyte regulation, osmolality, hematocrit, fat depletion, winter conditions, green sunfish, largemouth bass, brook trout, yellow perch, sodium, chloride

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Dans des expériences de laboratoires, des jeunes de l'année de crapet vert, *Lepomis cyanellus*, d'achigan à grande bouche, *Micropterus salmoides*, d'omble de fontaine, *Salvelinus fontinalis*, et de perchaude, *Perca flavescens*, furent maintenus durant 4 mois dans des conditions hivernales simulées (2–4°C; photopériode de 9 h de clarté : 15 h de noirceur) dans des solutions de pouvoir osmotique varié, 90% eau distillée à 1,0% NaCl. Dans trois sur cinq essais, la mortalité est plus élevée dans des solutions de sel, signe que NaCl ne favorise pas la survie. Cette dernière est plus élevée chez les grandes perchaudes que chez les petites, alors que chez les autres espèces, elle n'est pas liée à la taille. L'hématocrite, à la fin des expériences, avait baissé à des niveaux inférieurs à ceux des témoins dans toutes les solutions. En général, les poissons peuvent régler la concentration osmotique du plasma dans toutes les solutions, sauf celles des plus hautes concentrations de NaCl, où l'osmolalité plasmatique est souvent nettement supérieure à celle des témoins à la fin des expériences. Typiquement, le Na^+ et Cl^- plasmatique diminuent, pour ensuite se rétablir aux basses températures. La présence de Na^+ et Cl^- dans le milieu extérieur tend à alléger leur perte du plasma. Les changements de Na^+ et de Cl^- plasmatique ne sont pas liés à la mortalité dans des solutions hypoioniques, mais correspondent ordinairement aux changements d'osmolalité plasmatique. La teneur en gras des survivants diminue et la teneur en eau augmente chez toutes les espèces, mais les pertes

[1]Based on a thesis submitted by M.L.T. in partial fulfillment of the requirements for the degree of Master of Science from the University of Wisconsin, Stevens Point, WI 54481, USA.

[2]Present address: Ichthyological Associates, Inc., 100 S. Cass St., Middletown, DE 19709, USA.

[3]U.S. Fish and Wildlife Service, Wisconsin Department of Natural Resources, and University of Wisconsin (Stevens Point) cooperating.

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et les gains ne sont pas liés à l'osmolalité des solutions. La faible teneur en gras n'est probablement pas une cause de mortalité.

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AN overwinter increase in the mean length of samples of young-of-the-year has been observed for several species of fish (Toneys and Coble 1979). The increase was due to greater mortality of the small individuals than the larger — and not to growth (Lindroth 1965; Forney 1966; Negonovskaya 1972; Chevalier 1973) nor, in some cases, to predation (Hunt 1969; Toneys 1977; Oliver et al. 1979). Hunt (1969) suggested that inability to cope with "temperature-associated" stresses may cause such mortality.

At low temperatures fish may have reduced ability to iono-osmoregulate because of changes in membrane permeability and the functioning of ion transport mechanisms (Morris and Bull 1968; Stanley and Colby 1971; Davis and Simco 1976). Because electrolyte regulation consumes energy (Parvatheswararao 1970; Nordlie and Leffler 1975), the proportion of energy used for such regulation may be higher in low than in high temperatures and consequently may be an additional drain on fat stores.

The above considerations led to our two hypotheses: 1) that small young-of-the-year freshwater fish, which have lower fat reserves than larger fish, die during winter because of inadequate ionic–osmostic regulation, and 2) that survival would increase if small fish were held during winter in salt solutions approximately isosmotic with their blood.

We conducted laboratory experiments, simulating winter temperature and photoperiod, in an effort to relate ionic–osmotic imbalance or fat depletion to fingerling mortality.

Materials and Methods

GENERAL

Experiments were conducted with laboratory-raised green sunfish, *Lepomis cyanellus*, total length 23–50 mm; and with three species obtained from the Lake Mills (Wisconsin) National Fish Hatchery: largemouth bass, *Micropterus salmoides*, 50–90 mm (two experiments); brook trout, *Salvelinus fontinalis*, 52–150 mm; and yellow perch, *Perca flavescens*, 32–101 mm. Fish were held for 3 wk before experiments in circular fiberglass tanks containing 2190 L of flowing charcoal-filtered tap water. Temperature was regulated with heaters, and air stones provided aeration. Fish were fed dry pelleted trout food.

In each experiment, groups of fish were held for 100–120 d at simulated winter temperatures and photoperiod in three to five solutions ranging from 90% distilled:10% tap water to 1.0% NaCl. For each test 10–50 fish of a species were transferred from the holding tank to each of several bioassay jars (39 L) or glass aquaria (55 or 117 L) containing one of the solutions, where they remained at a constant temperature of 13–26°C depending on species, for a 1-wk acclimation period during which unhealthy fish were removed (Table 1). The rest of the fish in the holding tank served as a control group and were held at 13–26°C.

After the acclimation period, the experiments began when water temperature was decreased 1°C/d until 4°C was reached, and photoperiod was reduced gradually from 15 h light: 9 h dark to 9 h light:15 h dark. After about 3 wk at 4°C, water temperature was reduced to 2°C over a 2-d period, where it remained for about 2 mo until the conclusion of each experiment.

Feeding of trout pellets and assorted invertebrates was discontinued at temperatures below 10°C. Jars and aquaria were cleaned daily through partial solution changes while food was offered, and weekly thereafter. Salt solutions were made by mixing commercial-grade NaCl with chlorine-free tap water (pH 7.7; conductivity 330 mmhos; and [in mg/L] alkalinity 140, total hardness 178, Ca^{++}108, Cl^{-}7.5, Na^{+}3.0, orthophosphate 0.005, total phosphate 0.009, NH_4 nitrogen 0.14, NO_3–NO_2 nitrogen 0.25). Standing water in bioassay jars and glass aquaria was aerated and circulated through filters of activated charcoal and filter floss.

Fish were checked at least twice daily. Dead fish were removed and frozen for later determination of total body fat and water content.

BLOOD ANALYSIS

For all species except green sunfish (observations on which were confined to mortality), blood was collected once only from 5 to 10 fish from each solution, including the control group, at four times: at the start of the experiment, after fish were held at 4°C and at 2°C, each for at least 3 wk; and at the end of the experiment (2°C). A further exception was in the experiment with yellow perch; they were held at 4°C for only 9 d before the second blood sampling. Fish of different lengths were removed from their solution and measured to the nearest millimetre without anesthesia. Then the caudal peduncle was severed and the free-flowing blood was collected in heparinized microhematocrit tubes, which were sealed with clay sealer and centrifuged for 4 min at 10 500 rpm. After hematocrit determination, the section of tube containing red cells was discarded and the plasma was sealed in the remainder of the tube and stored at −12°C for later determinations of osmolality and sodium and chloride ion concentration. Fish were killed between 8:00 and 12:00 and were not fed for the previous 24 h.

Plasma from two or three yellow perch, but not from the other two species, was pooled after determination of hematocrit. Osmolality was measured by freezing point depression according to the apparatus and procedure of Ramsay and Brown (1955). Concentration of Na^{+} was determined by flame photometry and concentration of Cl^{-} by the method of Shales and Shales (1941), both on 5-μL samples of plasma diluted with 2 mL of deionized-distilled water. The plasma collected was insufficient for duplicate determinations on all samples.

BODY FAT AND WATER ANALYSIS

Mean body fat and water content were determined for the two lots of largemouth bass, and for brook trout and yellow perch. In the largemouth bass I experiment, base-

TABLE 1. Stocking densities, number of replicates, percent mortality, and mean lengths (mm) ± SD of four species held in various solutions for 9–53 d at 4°C followed by 75–87 d at 2°C. (*n*) is the number of fish at the beginning of each experiment.

Species, density, replicates	Solution[a]	Mortality % (*n*)	Mean length ± SD (mm)		*P*[†]
			Survivors	Dead	
Green sunfish	Distilled	9 (44)[c]	34±6	35±9	NS
15 fish/jar[b]	Tap	0 (45)	34±5	—	
3 jars/solution	0.1	2 (44)	34±4	38±0	
	0.5	0 (45)	33±5	—	
	1.0	79 (42)	34±3	33±4	NS
Largemouth bass I	Distilled	5 (44)	61±4	60±1	NS
10 fish/jar[b]	Tap	5 (43)	62±5	56±8	NS
6 jars/solution	0.1	2 (43)	61±4	57±0	—
	0.5	20 (49)	62±4	59±3	NS
	1.0	95 (43)	67±2	62±5	NS
Largemouth bass II	0.2	0 (29)	69±6	—	
11 fish/jar[b]	0.4	0 (31)	70±7	—	
5 jars/solution	0.6	0 (34)	67±6	—	
Brook trout	Distilled	0 (39)	80±7	—	
35 fish/aquarium[d]	Tap	0 (38)	77±10	—	
2 aquaria/solution	0.2	0 (46)	82±7	—	
	0.4	0 (44)	80±8	—	
	0.85	0 (43)	78±8	—	
Yellow perch	Distilled	37 (75)	48±4	43±4	<0.01
50 fish/aquarium[d]	Tap	55 (80)	48±3	42±4	<0.01
2 aquaria/solution	0.2	76 (77)	46±4	42±3	<0.01
25 fish/aquarium[e]	0.4	75 (100)	51±4	43±4	<0.01
1 aquarium/solution	0.6	82 (51)	49±2	45±4	<0.01

[a]Solutions: 90% distilled, 10% tap water; tap water; % NaCl.
[b]Jars held 39 L.
[c]The numbers of fish at the beginning of experiments are less than the numbers indicated by the replicates because fish that were removed during experiments and sacrificed for blood analysis and fish that died during the acclimation period were excluded from *n*.
[d]Aquaria held 117 L.
[e]Aquaria held 55 L.
[†]*t*-test.

line mean fat content was determined from a group of 25 fish of various lengths from all solutions, which were killed 1 d before temperature decrease began, and measured to the nearest millimetre, weighed to the nearest milligram, placed in water in plastic bags, and frozen at −20°C. In each of the other experiments, groups of 15–40 fish were killed after the decreasing water temperature reached 10°C, and measured, weighed, and frozen. At the end of the experiments, groups of 15–20 fish from each solution also were frozen for fat and water determination.

After storage, individual fish were placed on aluminum planchets and oven-dried for 96 h at 70°C. Fish weighing 4 g or less (wet weight) were dried intact; larger fish were cut in two or more pieces before drying. After drying, the planchets containing fish were cooled in a desiccator for at least 2 h. Dry weight was then determined and percent total body water was calculated.

Dried fish of 0.2 g or heavier were ground in a Thomas Wiley Mill with a 2-mm screen. Fat was extracted from duplicate subsamples of 0.05–0.2 g with Freon 11 (monofluorotrichloromethane) for 1 h (Korn and Macedo 1973). Dried fish lighter than 0.2 g were cut into small pieces but not ground before fat extraction. We modified the extraction procedure for the small fish because the samples were so small; anhydrous sodium sulfate was reduced from 80 to 40 g per sample, and Freon 11 was reduced from 100 to 40 mL per sample. We found through previous testing that these reductions did not affect the extraction.

Results and Discussion

MORTALITY

No strong relation between survival and size was evident in this study. Fish died in three of the five experiments (green sunfish, largemouth bass I, and yellow perch) and mortality was much higher for yellow perch than for the other species (Table 1). Among the fusiform fish, i.e. excluding green sunfish, yellow perch were the smallest, and only for yellow perch were survivors significantly larger than those that died (Table 1). The lack of well-defined, size-related mortality among the other species is consistent with the con-

TABLE 2. Osmolality and sodium and chloride ion concentrations (mmol/L) of nine experimental solutions

Solution	Osmolality	Sodium	Chloride
90% Distilled:10% tap	16	1<	1<
Tap water	22	1<	1<
NaCl solutions (%)			
0.1	38	16	23
0.2	81	35	46
0.4	172	61	89
0.5	215	93	105
0.6	242	108	126
0.85	323	156	178
1.0	361	183	198

clusion of Toneys and Coble (1979). After reviewing investigations of size-related, first winter mortality of freshwater fish, they concluded that although such mortality has been found for several species, including largemouth bass and brook trout, it does not seem to be a general phenomenon for some species, including largemouth bass.

The small size of yellow perch compared to largemouth bass and brook trout in this study suggests that there may be thresholds of size (length, weight, stored energy) below which size-related deaths occur and above which they do not. Oliver et al. (1979) suggested a similar phenomenon. The values of such thresholds could be expected to vary with environmental conditions, i.e. adequate size or condition in one set of circumstances might not be adequate in others. If real, such thresholds could help explain the inconsistent appearance of size-related mortality that has been found (Toneys and Coble 1979).

Patterns of mortality during the experiments did not support the hypothesis that hyposmotic salt solutions approaching the concentration of fish blood (0.6–0.85% NaCl) would reduce mortality of small fish at low temperatures. Generally mortality was greater in salt than in nonsalt solutions (Table 1) and among yellow perch, mortality increased with increasing NaCl concentration, indicating that presence of salt in the external medium was not beneficial and may have been detrimental. The 1.0% NaCl solution was hyperosmotic to the blood of largemouth bass (Tables 2 and 3) and probably green sunfish, and in these solutions mortalities were among the highest observed, a result also found by Ahokas and Sorg (1977) for the banded killifish, *Fundulus diaphanus*.

These results agree with unpublished data of Coble in Appendix A of Toneys (1977), which showed that mortality of young-of-the-year largemouth bass was greater in hyposmotic salt solutions than in tap water during winter, and with those of Pitkow (1960) and Stanley and Colby (1971) who found that increased salinity did not increase survival of the guppy, *Poecilia reticulata*, and alewife, *Alosa pseudoharengus*, in cold water.

HEMATOCRIT

Hematocrit usually declined with decrease in water temperature, but low hematocrit apparently was neither a cause of mortality nor a function of salt concentration (Table 3). The decrease appeared to be a general response to drop in temperature or food deprivation, or both, because it occurred in all solutions. Hematocrit was significantly lower than control values at the end of both largemouth bass experiments and the brook trout experiment, but differences were not significant in most solutions in the yellow perch experiment, where mortality was highest.

The effect of decreased temperature as opposed to food deprivation on lowered hematocrit is not clear from earlier studies. Several authors found hematocrit to decrease in starved fish (Kamra 1966 with Atlantic cod, *Gadus morhua*; Ince and Thorp 1976 with northern pike, *Esox lucius*; J. G. Stanley, Maine Cooperative Fishery Research Unit, Department of Zoolgy, University of Maine, Orono, ME 04473, USA, personal communication, with the plains killifish, *Fundulus kansae*), but others have found no such decrease in starved European eel, *Anguilla anguilla* (Larsson and Lewander 1973; Johansson-Sjöbeck et al. 1975). Kristoffersson et al. (1972) stated that after a sudden temperature drop of 10°C, hematocrit decreased in northern pike that had not fasted, and that at 6°C the fasting effect observed at 16°C could not be demonstrated. Houston and De Wilde (1969) related decrease in brook trout hematocrit to the decreased blood O_2 carrying capacity required in cold water.

PLASMA OSMOLALITY

All three species generally were able to maintain plasma osmotic concentration at low temperatures without the aid of hyposmotic or isosmotic salt solutions (Table 3). Fewer significant changes in osmolality occurred in the non-salt solutions than in those containing NaCl. In the non-salt solutions there were usually only small differences in osmolality between control and experimental groups at the end of experiments.

Plasma osmolality was highest at the end of experiments in the highest salt concentrations. Osmolality increased steadily in some cases (largemouth bass I, brook trout) but initially decreased before rising in others (largemouth bass II, yellow perch).

These results indicate that the temperate species tested are able to regulate plasma osmolality satisfactorily in freshwater at low temperatures. Allanson et al. (1971) found the opposite for the cichlid, *Tilapia mossambica*. In that species plasma osmolality decreased at low temperature in freshwater but not in brackish water.

PLASMA SODIUM AND CHLORIDE

The general response of plasma sodium and chloride ion concentration to low temperature was an initial de-

TABLE 3. Mean ± SD, (*n*), hematocrit (%), and plasma osmolality (mmol/L). Number of days held before blood sampling at 4 and 2°C and at the end (2°C) of experiments was 21, 23, and 58, respectively, for largemouth bass I; 25, 21, and 64 for largemouth bass II; 23, 22, and 65 for brook trout; and 9, 21, and 65 for yellow perch.

Species and solution	Hematocrit (%)				Osmolality (mmol/L)			
	Start	4°C	2°C	End (2°C)	Start	4°C	2°C	End (2°C)
Largemouth bass I								
Control[a]	39±3 (5)	30±2 (5)	24±4 (5)	36±3 (5)	301±10 (5)	312± 7 (5)	308±12 (5)	309± 6 (5)
Experimental								
90% distilled water[b,c]	36±2 (5)	17±3 (5)	20±6 (5)	25±5 (5)[d]	297± 7 (5)	301±13 (5)	294±10 (5)	305± 7 (5)
Tap water	36±7 (5)	11±2 (5)	22±6 (5)	23±4 (5)[d]	309± 3 (5)	290±12 (4)	290±10 (5)	309±12 (5)
NaCl solutions (%)								
0.1[c]	39±3 (5)	17±3 (5)	19±3 (5)	19±3 (5)[d]	308±14 (5)	317±12 (4)	332±10 (5)	323±10 (5)[e]
0.5[b]	37±1 (5)	20±5 (3)	19±2 (5)	26±5 (5)[d]	300±10 (5)	328±19 (3)	332±13 (5)	366±19 (5)[d]
1.0[c]	31±4 (5)	19±1 (4)	26±3 (5)	20±6 (3)[d]	323±15 (5)	344±10 (4)	359± 8 (5)	398±19 (3)[d]
Largemouth bass II								
Control[a]	36±2 (5)	41±7 (5)	33±5 (5)	36±7 (5)	332± 8 (5)	315±11 (5)	314±16 (5)	361± 5 (5)
Experimental								
NaCl solutions (%)								
0.2	41±8 (5)	27±4 (5)	25±3 (5)	21±3 (5)[d]	325±21 (5)	323± 4 (5)	318± 6 (5)	337± 6 (5)[d]
0.4	45±6 (5)	27±4 (5)	27±3 (5)	20±2 (5)[d]	340±14 (5)	314± 5 (5)	339± 5 (5)	342± 6 (5)[d]
0.85[b]	33±7 (5)	23±3 (5)	25±3 (5)	21±3 (5)[d]	347±19 (5)	322±15 (5)	373± 8 (5)	318±49 (5)
Brook trout								
Control[a]	37±5 (5)	37±4 (5)	35±4 (5)	35±4 (5)	338±12 (5)	323±16 (5)	339±11 (5)	318± 5 (5)
Experimental								
90% distilled water[b]	35±4 (5)	27±3 (5)	24±3 (5)	23±4 (5)[d]	347±15 (5)	332±16 (5)	338±10 (5)	324±14 (5)
Tap water[b,c]	33±3 (5)	24±4 (5)	28±3 (5)	20±5 (5)[d]	353±25 (5)	342±13 (5)	343±10 (5)	333±14 (5)[e]
NaCl solutions (%)								
0.2[b]	34±3 (5)	27±2 (5)	24±2 (5)	15±3 (5)[d]	350±16 (5)	341±11 (5)	339±11 (5)	356±14 (5)[d]
0.4[b]	34±3 (5)	24±1 (5)	24±2 (5)	14±2 (5)[d]	333±16 (5)	342± 6 (5)	358± 8 (5)	339±23 (5)
0.85[b]	32±3 (5)	27±2 (5)	27±4 (5)	17±2 (5)[d]	327±20 (5)	348±10 (5)	358±11 (5)	373± 8 (5)[d]
Yellow perch								
Control[a]	42±7 (6)	32±2 (6)	33±3 (6)	27±2 (5)	299±21 (5)	280±21 (5)	281± 9 (5)	326±12 (5)
Experimental								
90% distilled water[b]	34±2 (6)	29±2 (5)	31±3 (6)	24±5 (8)	320±53 (5)	196± 8 (5)	218±16 (5)	325±15 (5)
Tap water	32±3 (6)	25±3 (5)	30±2 (5)	22±4 (8)	351±37 (5)	208±16 (5)	226±15 (5)	326±15 (5)
NaCl solutions (%)								
0.2	37±4 (6)	28±2 (6)	23±5 (6)	26±7 (7)	346±15 (5)	210± 5 (5)	238±21 (5)	332±16 (5)
0.4	31±3 (6)	32±2 (7)	31±5 (5)	18±2 (7)[d]	332±21 (5)	195± 7 (5)	234±23 (5)	304±10 (5)[e]
0.6	32±5 (6)	37±6 (6)	31±4 (6)	23±7 (5)	304±36 (5)	223±16 (5)	225±36 (5)	362±12 (4)[d]

[a]Temperatures in the headings do not apply to the controls, which were held throughout the experiments at 14–21°C (largemouth bass), 13°C (brook trout), and 18°C (yellow perch). Control and experimental fish were sampled at the start and end of the experiment and on the same two occasions during the experiment.

[b,c]Experimental groups differed from control during experiment (paired *t*): (b) hematocrit, $P < 0.05$; (c) osmolality, $P < 0.05$.

[d,e]Experimental group differed from control at end (*t*-test): (d) $P < 0.01$; (e) $P < 0.05$.

crease in all solutions, followed by an increase to near starting concentrations or to higher levels in hyperionic solutions (Table 4). Sodium and chloride ions in the external medium seemed to alleviate loss of these ions from plasma, as suggested by Kristoffersson et al. (1972), but the evidence was not unequivocal because expected trends, e.g. more marked changes in the lower salt solutions, were not consistent in the experiments.

There was no apparent relation between changes in plasma Na^+ and Cl^- and mortality (Tables 1, 4). Sometimes a significant change in plasma ion concentration occurred in a group that suffered no mortality (brook trout in 0.85% NaCl), and high mortality occurred when a significant change in plasma ion concentration was not detected (yellow perch in 0.6% NaCl solution).

Conversely, experimental groups that differed significantly from controls for osmolality usually also differed in plasma ion concentrations. Significant differences in plasma electrolyte concentration occurred in 8 of 11 experimental groups in which significant differences occurred in osmolality (Tables 3, 4). Corresponding changes in plasma osmolality and electrolytes were also found by Gordon (1963), Houston and Madden (1968), and Davis and Simco (1976); however, the closeness of the correspondence may be expected to vary with species (Umminger 1971).

Although it often does not lead to death, a decrease in Na^+ and Cl^- in blood of fish held in cold water is a general phenomenon (Umminger 1971), for it has been observed in many studies (Wikgren 1953; Meyer et al. 1956; Heinicke and Houston 1965; Toews and Hickman 1969; Allanson et al. 1971). The pattern of initial decrease followed by an increase in electrolytes was also found by Reaves et al. (1968) and Kristoffersson et al. (1972). Prosser et al. (1970) viewed the decrease as an adaptive mechanism directed at reduction of osmotic work through a decrease in the osmotic gradient.

BODY FAT AND WATER CONTENT

The pattern of fat depletion did not support the hypothesis that less energy, in the form of fat, would be used if fish were held in salt solutions of osmotic concentration approaching that of fish blood. Fat content of survivors declined 13.2–41.0%, and water con-

TABLE 4. Mean ± SD, (*n*) plasma sodium and chloride ion concentrations (mmol/L). Number of days held before blood sampling at 4 and 2°C and at the end (2°C) of experiments was 21, 23, and 58, respectively, for largemouth bass I; 25, 21, and 64 for largemouth bass II; 23, 22, and 65 for brook trout; and 9, 21, and 65 for yellow perch.

Species and solutions	Sodium (mmol/L)				Chloride (mmol/L)			
	Start	4°C	2°C	End (2°C)	Start	4°C	2°C	End (2°C)
Largemouth bass I								
Control[a]	—	—	—	—	91 ± 8 (5)	104 ± 6 (4)	100 ± 15 (5)	116 ± 4 (5)
Experimental								
90% distilled water	—	—	—	—	95 ± 7 (5)	80 ± 8 (5)	84 ± 6 (5)	78 ± 8 (5)[b]
Tap water	—	—	—	—	99 ± 10 (5)	83 ± 5 (4)	81 ± 10 (5)	97 ± 9 (5)[b]
NaCl solutions (%)								
0.1[e]	—	—	—	—	89 ± 13 (5)	93 ± 17 (4)	97 ± 11 (5)	108 ± 12 (5)
0.5	—	—	—	—	106 ± 7 (5)	118 ± 11 (3)	100 ± 10 (5)	109 ± 21 (5)
1.0	—	—	—	—	112 ± 19 (5)	105 ± 14 (3)	144 ± 6 (5)	140 ± 18 (3)[b]
Largemouth bass II								
Control[a]	137 ± 9 (5)	137 ± 15 (5)	143 ± 11 (5)	133 ± 7 (5)	127 ± 10 (5)	123 ± 3 (5)	118 ± 4 (5)	114 ± 9 (5)
Experimental								
NaCl solutions (%)								
0.2	151 ± 13 (4)	138 ± 16 (4)	136 ± 14 (5)	141 ± 3 (4)	110 ± 9 (5)	112 ± 8 (5)	112 ± 5 (5)	125 ± 10 (5)
0.4	132 ± 18 (4)	151 ± 8 (5)	148 ± 5 (4)	138 ± 5 (4)[d]	126 ± 13 (5)	109 ± 4 (5)	120 ± 13 (5)	135 ± 15 (5)
0.85	142 ± 16 (4)	153 ± 7 (5)	157 ± 2 (4)	125 ± 21 (4)	130 ± 22 (5)	120 ± 19 (5)	135 ± 7 (5)	109 ± 31 (5)
Brook trout								
Control[a]	139 ± 13 (5)	134 ± 15 (5)	135 ± 14 (5)	142 ± 13 (5)	120 ± 9 (5)	120 ± 5 (5)	129 ± 8 (5)	132 ± 7 (5)
Experimental								
90% distilled water	148 ± 7 (5)	113 ± 5 (5)	139 ± 25 (5)	138 ± 11 (5)	127 ± 6 (5)	115 ± 15 (5)	119 ± 23 (5)	134 ± 4 (5)
Tap water[c]	126 ± 9 (5)	115 ± 12 (5)	133 ± 19 (5)	134 ± 10 (4)	123 ± 13 (5)	118 ± 8 (5)	132 ± 7 (5)	126 ± 18 (5)
NaCl solutions (%)								
0.2[f]	137 ± 12 (5)	119 ± 6 (5)	133 ± 10 (4)	127 ± 7 (4)	131 ± 6 (5)	126 ± 14 (5)	138 ± 4 (4)	139 ± 4 (5)
0.4	137 ± 7 (5)	128 ± 15 (4)	132 ± 21 (5)	127 ± 8 (5)	125 ± 9 (5)	119 ± 18 (5)	149 ± 12 (5)	126 ± 24 (5)
0.85	132 ± 14 (4)	140 ± 7 (4)	142 ± 16 (4)	162 ± 23 (4)	131 ± 16 (5)	140 ± 13 (5)	132 ± 11 (5)	162 ± 3 (5)[b]
Yellow perch								
Control	119 ± 23 (5)	141 ± 9 (5)	139 ± 19 (5)	134 ± 4 (5)	106 ± 6 (5)	—	—	109 ± 4 (5)
Experimental								
90% distilled water	127 ± 23 (5)	116 ± 14 (5)	98 ± 13 (5)	117 ± 7 (5)	114 ± 7 (5)	—	—	100 ± 4 (5)[d]
Tap water	133 ± 17 (5)	114 ± 5 (5)	118 ± 10 (5)	124 ± 6 (5)[d]	110 ± 2 (5)	—	—	89 ± 9 (5)
NaCl solutions (%)								
0.2	133 ± 8 (5)	106 ± 12 (5)	109 ± 8 (5)	124 ± 5 (5)[d]	125 ± 9 (5)	—	—	89 ± 4 (5)
0.4[e]	114 ± 16 (5)	125 ± 3 (5)	128 ± 20 (5)	120 ± 12 (5)	123 ± 7 (5)	—	—	101 ± 7 (5)
0.6	138 ± 24 (5)	123 ± 16 (5)	125 ± 20 (5)	136 ± 2 (4)	129 ± 15 (5)	—	—	112 ± 15 (4)

[a]Temperatures in the headings do not apply to the controls, which were held throughout the experiments at 14–21°C (largemouth bass), 13°C (brook trout), and 18°C (yellow perch). Control and experimental fish were sampled at the start and end of the experiment and on the same two occasions during the experiment.

[b,c]Experimental group differed from control: (b) at end of experiment (*t*-test, $P < 0.01$); (c) chloride during experiment (*t*-test, $P < 0.05$).

[d]Experimental group differed from control at end (*t*-test, $P < 0.05$).

[e,f]Experimental group differed from control during the experiment (paired *t*): (e) sodium, $P < 0.05$; (f) chloride, $P < 0.01$.

TABLE 5. Mean ± SD, (*n*) percent fat (dry weight) at the beginning and end of experiments in which fish were held under simulated winter conditions for 100–120 d in different solutions. See Tables 3 or 4 for holding temperatures.

Stage of experiment, and medium	Largemouth bass		Brook trout	Yellow perch
	Experiment 1	Experiment 2		
Start	8.1 ± 2.4 (25)	15.5 ± 2.8 (16)	17.3 ± 3.0 (15)	6.5 ± 3.3 (39)
Conclusion				
Control	11.9 ± 1.1 (15)	15.8 ± 2.6 (15)	16.4 ± 2.8 (9)	10.6 ± 1.5 (12)
90% distilled water	4.8 ± 1.5[a] (13)	—	13.9 ± 3.6[b] (12)	4.2 ± 0.4[b] (16)
Tap water	5.0 ± 2.6[a] (13)	—	11.5 ± 3.2[a] (12)	3.9 ± 0.4[a] (14)
NaCl solutions (%)				
0.1	3.8 ± 1.7[a] (15)	—	—	—
0.2	—	12.5 ± 2.5[a] (13)	13.2 ± 1.9[a] (11)	4.0 ± 0.5[b] (10)
0.4	—	14.5 ± 1.9 (15)	12.3 ± 3.4[a] (12)	4.3 ± 1.1[b] (12)
0.5	5.6 ± 1.1[a] (14)	—	—	—
0.6	—	—	—	4.4 ± 0.8 (9)
0.85	—	13.3 ± 2.5[b] (14)	12.2 ± 3.1[a] (12)	—

[a,b]Experimental value differed from starting value (*t*-test): (a) $P < 0.01$; (b) $P < 0.05$.

TABLE 6. Mean ± SD, (*n*) percent total body water at the beginning and end of experiments in which fish were held under simulated winter conditions for 100–120 d in different solutions. See Tables 3 or 4 for holding temperatures.

Stage of experiment, and medium	Largemouth bass		Brook trout	Yellow perch
	Experiment 1	Experiment 2		
Start	78.7±1.2 (25)	75.5±1.1 (16)	79.8±1.5 (15)	83.3±1.3 (39)
Conclusion				
Control	75.6±0.4 (15)	76.6±1.3 (15)	77.4±0.9 (9)	75.9±1.3 (12)
90% distilled water	81.8±0.7[a] (13)	—	83.0±1.6[a] (12)	82.7±1.2 (16)
Tap water	80.7±0.9[a] (13)	—	82.9±1.4[a] (12)	83.4±1.3 (14)
NaCl solutions (%)				
0.1	81.2±0.8[a] (15)	—	—	—
0.2	—	80.4±1.5[a] (13)	81.6±1.6[a] (11)	84.8±2.0[a] (10)
0.4	—	78.0±1.2[a] (15)	82.0±1.2[a] (12)	83.8±1.2 (12)
0.5	81.3±0.6[a] (14)	—	—	—
0.6	—	—	—	84.3±1.3[b] (9)
0.85	—	78.9±2.4[a] (14)	81.7±0.8[a] (12)	—

[a,b]Experimental value differed from starting value (*t*-test): (a) $P < 0.01$; (b) $P < 0.05$.

tent increased 0.6–4.7%, for all three species (Tables 5, 6), but neither fat loss nor water gain were related to osmolality of the solutions. Rather, water gain appeared to be a function of fat loss, a relation observed in other studies, but one that can be misleading (Meyer et al. 1956; Stanley 1969; Caulton and Bursell 1977).

Fat loss may have been related to mortality in yellow perch. The yellow perch experiment was the only one in which substantial mortality occurred in all hyposmotic solutions. Fat content of survivors was always higher than that of perch that died, and the difference was significant in three of the five solutions (Table 7). However, dead yellow perch had more than 3% fat; Newsome and LeDuc (1975) believed that 2% fat was the critically low level for yellow perch. Oliver et al. (1979) also found that lipid declined under winter conditions in smallmouth bass, *Micropterus dolomieui*, but reduced lipid content did not seem to be related to death.

There was a trend toward higher fat content in the larger fish that survived for all species, but correlation coefficients between fat content and length of fish, which ranged from 0.03 to 0.61, were seldom significant. The trend was reversed for water content; larger fish had a lower proportion of water, and correlations, which were frequently significant, ranged from −0.18 to −0.79.

The amount of energy needed for osmoregulation is not clearly understood. Our lack of a relation between fat depletion (a measure of energy expenditure) and salinity contrasts with results of Rao (1968) and Farmer and Beamish (1969) who found decreased oxygen consumption (a measure of energy expenditure) in fish held in isomotic solutions. Our results are consistent with the suggestion of Gordon (1973) that different salinities would not require more or less osmotic work, but rather a simple change in gill membrane permeability. Wikgren (1953) concluded from a review of several studies that the amount of energy needed for active ion absorption is a small fraction of total metabolism; Parvatheswararao (1970) suggested that that may be the case; and Spaargaren (1975) reached essentially the same conclusion regarding osmoregulation in hyposmotic media. If so, gross fat depletion may not have been adequately sensitive for detection of differences in energy expenditure in the various solutions.

TABLE 7. Mean ± SD, (*n*), percent fat (dry weight) of yellow perch that died and that survived.

Solution	Dead	Alive
90% Distilled water[a]	3.7±0.6 (20)	4.2±0.4 (15)
Tap water	3.5±0.9 (26)	3.9±0.4 (14)
NaCl solutions (%)		
0.2[b]	3.3±0.6 (25)	4.0±0.5 (11)
0.4	3.8±1.3 (26)	4.3±1.1 (12)
0.6[b]	3.3±0.8 (17)	4.4±0.8 (9)

[a,b]Values for dead and living fish significantly different (*t*-test): (a) $P < 0.05$; (b) $P < 0.01$.

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Effects on Rainbow Trout (*Salmo gairdneri*) of a Chronic Exposure to Waterborne Selenium

P. V. HODSON, D. J. SPRY, AND B. R. BLUNT

Department of Fisheries and Oceans, Great Lakes Biolimnology Laboratory, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6

HODSON, P. V., D. J. SPRY, AND B. R. BLUNT. 1980. Effects on rainbow trout (*Salmo gairdneri*) of a chronic exposure to waterborne selenium. Can. J. Fish. Aquat. Sci. 37: 233–240.

The 96-h and 9-d LC50s of selenium for rainbow trout (*Salmo gairdneri*) averaged 8.1 and 6.5 mg·L^{-1}, respectively. A sublethal exposure of rainbow trout from the newly fertilized egg stage until 44 wk posthatch to 4.4–53 μg·L^{-1} of waterborne selenium resulted in a variety of subtle responses. Significantly increased mortality of eyed eggs was observed at selenium concentrations greater than or equal to 28 μg·L^{-1} and decreased red blood cell volumes and cellular blood iron at concentrations greater than or equal to 53 and 16 μg·L^{-1}, respectively. Although the highest eyed egg mortality at 47 μg·L^{-1} selenium was double that of control, total mortality was only 6.5%. Similarly, the blood parameters decreased from control levels by a maximum of 30% and the fish appeared to be compensating for these changes by increased erythropoiesis. Overall, the fish responded to selenium exposure at concentrations between 100 and 250 times the normal background concentrations in surface waters but the level of response was low. Analyses of tissue selenium at 44 wk posthatch indicated that residues clearly reflected exposure, that liver, digestive tract, and kidney accumulated the highest residues, that selenium tissue concentrations may be regulated, and that specific tissues provide a better basis for monitoring selenium exposure than whole body analyses.

Key words: selenium, toxicity, fish, chronic, rainbow trout

HODSON, P. V., D. J. SPRY, AND B. R. BLUNT. 1980. Effects on rainbow trout (*Salmo gairdneri*) of a chronic exposure to waterborne selenium. Can. J. Fish. Aquat. Sci. 37: 233–240.

La CL50 après 96 h et 9 d du sélénium pour la truite arc-en-ciel (*Salmo gairdneri*) est en moyenne de 8,1 et 6,5 mg·L^{-1} respectivement. Une exposition sublétale de truites arc-en-ciel à des stades allant de l'oeuf nouvellement fécondé à 44 semaines après éclosion à 4,4–53 μg·L^{-1} de sélénium porté par l'eau entraîne une variété de réponses subtiles. A des concentrations de sélénium supérieures ou égales à 28 μg·L^{-1}, on observe des mortalités nettement plus élevées d'oeufs embryonnés, alors qu'à des concentrations supérieures ou égales à 53 et 16 μg·L^{-1}, il y a diminution de volume d'érythrocytes et de fer sanguin cellulaire respectivement. Bien que la mortalité la plus élevée d'oeufs embryonnés à 47 μg·L^{-1} de sélénium soit le double de celle des témoins, la mortalité totale n'est que de 6,5%. De même, les paramètres du sang diminuent par rapport aux niveaux témoins d'un maximum de 30%, et les poissons semblent compenser ces changements par une érythropoïèse accrue. Dans l'ensemble, les poissons réagissent à une exposition au sélénium à des concentrations de 100 à 250 fois plus fortes que les concentrations normales naturelles des eaux de surface, mais la réaction est faible. Le dosage du sélénium tissulaire chez les alevins de 44 semaines après éclosion indique que les résidus reflètent clairement l'exposition, que le foie, le tractus digestif et le rein accumulent le plus de résidus, que les concentrations de sélénium dans le tissu peuvent être réglées et enfin, que des tissus spécifiques permettent de mieux suivre l'exposition au sélénium que des analyses du corps entier.

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THIS paper demonstrates that rainbow trout (*Salmo gairdneri*) respond physiologically to waterborne selenium at concentrations less than or equal to 53 μg·L^{-1}. However, the low level of these responses suggests little harm to trout during prolonged exposures.

Selenium may enter the Great Lakes from manufacturing of electronic equipment, steel, and pigments, as well as from combustion of fossil fuels (I.J.C. 1976). Copeland (1970) found that elevated concentrations of selenium in Lake Michigan zooplankton corresponded to dispersion patterns of air pollution from industrial areas on the western shore. A water quality objective for the Great Lakes of 10 μg·L^{-1} has been adopted by the International Joint Commission (I.J.C. 1977) to protect raw waters for public water supplies. However, the rationale for this objective considered very little data on the sublethal effects of selenium on aquatic biota (I.J.C. 1976). Lethal concentrations for fish vary from about 2–80 mg·L^{-1}, depending upon the selenium

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salt or the fish species tested (Niimi and LaHam 1975; Ellis et al. 1937; Weir and Hine 1970; Cardwell et al. 1976). One study of sublethal effects of selenium toxicity was published prior to 1976 indicating impaired learning behaviour of goldfish (*Carassius auratus*) after short-term exposure to 250 $\mu g \cdot L^{-1}$ selenium (Weir and Hine 1970). This study was undertaken to determine whether chronic exposure to low concentrations of waterborne selenium would affect fish.

Materials and Methods

Design

Rainbow trout (*Salmo gairdneri*) were exposed from egg fertilization until 44 wk posthatch to nominal concentrations of 0, 5, 9, 16, 28, and 50 $\mu g \cdot L^{-1}$ selenium as sodium selenite. The six concentrations were randomly distributed among six 324-L tanks in each of three rows or replicates for a total of 18 tanks. Parameters measured were egg development rate, percent mortality of uneyed and eyed eggs, percent hatch, percent deformities, percent mortality of swim-up fry, fry and fingerlings, and growth rate. In addition, hematocrit, blood iron, red blood cell counts, plasma protein, plasma glucose, plasma osmolarity, and condition factor were measured 23 and 44 wk posthatch when the means (and standard deviations) of wet weights were 22.6 (9.7) and 181 (81) g, respectively. Selenium residues in tissues were measured at 44 wk posthatch. All results were subjected to analyses of variance and differences between means tested by Duncan's New Multiple Range Test (Steel and Torrie 1960).

The acute lethality of selenium to trout averaging 0.8 g wet weight was also measured by a 9-d bioassay following the methods of Sprague (1969). Nominal concentrations were 0, 2, 3.5, 6.3, 11.2, and 20 $mg \cdot L^{-1}$, temperature was 15°C, 10 fish per tank were tested and the bioassay was run in triplicate. During the bioassay, fish in the first replicate were fed a commercial diet at a ration of 6% of the body weight per day. In the second they were fed 6% every second day, and in the third they were not fed at all. The LC50s and 95% fiducial intervals were calculated by probit analysis (Finney 1971) using a computer program (BMD 032, Dixon 1971) based on the means of daily measurements of selenium concentration.

Water

The dilution water originated from Lake Ontario and was filtered and chlorinated as a municipal water supply and dechlorinated for laboratory use by charcoal filtration. A detailed chemical analysis is given in Table 1. Temperature during the egg exposure was 9.1 ± 0.5°C. During the posthatch exposure, the temperature was adjusted to an annual cycle to test whether temperature stress would enhance selenium toxicity. The temperature was adjusted from 9.1 to 24°C between March 5 and August 18, 1977, after which it was reduced to 11°C by October 20 and 7.5°C by January 20, 1978. Dissolved oxygen, conductivity, and pH were 8.2 ± 1.3 $mg \cdot L^{-1}$ (86 ± 13% saturation), 317 ± 26 $\mu mhos \cdot cm^{-1}$, and 7.89 ± 0.3, respectively. During the period of highest temperature, oxygen was maintained at about 8.4 mg/L (100% saturation).

TABLE 1. Chemical characteristics of the dilution water.

Parameter	$\bar{x}$[a]	SD	*n*
Hardness ($mg \cdot L^{-1}$)	135	2.5	50
Alkalinity ($mg \cdot L^{-1}$)	90	2.9	51
Calcium ($mg \cdot L^{-1}$)	41	1.1	13
Magnesium ($mg \cdot L^{-1}$)	8.1	0.3	13
Dissolved organic carbon ($mg \cdot L^{-1}$)	2.4	1.2	8
Fluoride ($\mu g \cdot L^{-1}$)	980	110	11
Nitrogen (nitrate + nitrite; $\mu g \cdot L^{-1}$)	570	260	9
Phosphorus, total ($\mu g \cdot L^{-1}$)	45	33	9
Iron ($\mu g \cdot L^{-1}$)	13	14	55
Arsenic ($\mu g \cdot L^{-1}$)	3.5	2.0	46
Zinc ($\mu g \cdot L^{-1}$)	2.9	2.7	53
Copper ($\mu g \cdot L^{-1}$)	2.7	2.5	48
Nickel ($\mu g \cdot L^{-1}$)	1.7	1.2	12
Chromium ($\mu g \cdot L^{-1}$)	1.3	1.8	52
Lead ($\mu g \cdot L^{-1}$)	1.1	1.2	53
Cadmium ($\mu g \cdot L^{-1}$)	<1		

[a] $\bar{x}$ = mean, SD = standard deviation, *n* = number of observations.

Diluter

Sodium selenite (Na_2SeO_3) was added to water as an alkaline stock solution by a doser (Hodson 1979) that fed a proportional diluter (Mount and Brungs 1967). The diluter delivered 1.75 L of solution at each concentration every 76 s. The flow was split three ways for the three replicates to give 460 mL/min per tank and each tank contained 216 L. At 15 wk posthatch, the flow rate was increased to 500 mL/min per tank to give a 95% molecular replacement time of 21 h (Sprague 1969). Water samples were taken daily for selenium analyses by atomic absorption spectrophotometry (Goulden and Brooksbank 1974). Responses of eggs were related to the measured concentrations during the egg exposure, while responses posthatch were related to the posthatch concentrations. The same diluter and flow rates were used for both chronic and acute bioassays.

Fish

Rainbow trout eggs from four ripe females were pooled and fertilized with milt from two males at Norfolk Trout Fisheries, Simcoe, Ontario. Within 3 h, 40 mL of eggs were added to hatching trays in each of the selenium exposure tanks to give from 204 to 275 eggs per tray. No chemical prophylactic treatments were used and dead eggs were removed at regular intervals to minimize fungal growth. The eggs were kept in the dark and a sample was removed every 2–5 d to check their development rate according to the criteria of Ballard (1973). During egg development, records were kept of the parameters indicated above (Design).

After hatch, a photoperiod of 16 h light, 8 h dark, was imposed. The lighting source was mercury halide ceiling lamps that gave 3.5–10.5 lx at the water surface in an exposed area of each tank, and 0.04–1.1 lx in a shaded area of each tank. The temperature was raised from 9.1°C as described, and the percent mortality of sac and swim-up fry recorded. After swim-up, the fry were fed a commercial trout starter by automatic feeders. To test whether diet

quality interacted with selenium, each replication of the six concentrations received a different brand of commercial fish feed. The rate of feeding and the granule size followed the recommendations of Martin's Feed Mills, Elmira, Ontario. The rate was the same for each replicate and was adjusted for temperature and average fish size based on weekly weight measurements. The frequency of feeding was reduced from 12 times daily at the swim-up stage to 2–3 times daily by the end of the experiment. The total biomass of fish in each tank was limited by the flow rate to 240–330 g based on a flow criteria of 2–3 L/g of fish per day (Sprague 1969). Consequently, fish were thinned when their average weight doubled, starting at 0.2 g. At each thinning, sufficient fish were removed to keep the total biomass within the criterion of 3 L/g of fish per day when the fish were small and had a high metabolic rate. After the fish achieved 12.8 g average weight, a criterion of 2 L/g of fish per day was adopted and the flow rate was increased at 15 wk posthatch to increase the allowable biomass to 360 g. Consequently, the number of fish tested was kept as high as possible. When the number of fish per tank was 10 or less, sampling biased the tank average weights sufficiently that growth data became meaningless and thinning was ad hoc to maintain 360 g total biomass.

Physiological Methods

Two fish per tank, for a total of six at each concentration, were randomly sampled at 23 wk posthatch at a temperature of 24°C. The sampling was repeated at 44 wk posthatch at a temperature of 7.5°C. At this time, only one fish per tank for a total of three fish per concentration was available for sampling. Condition factor (100 $W \cdot L^{-3}$) was calculated from the weight (W; g) and standard length (L; cm). Blood was sampled by caudal severance and hematocrit, red blood cell (RBC) counts, and blood iron ($\mu g \cdot mL^{-1}$ whole blood) were measured by the methods outlined by Hodson et al. (1978). Blood iron as ($\mu g \cdot mL^{-1}$ RBCs) was calculated by dividing the whole blood iron concentration by (hematocrit/100). Blood iron as ($\mu g \cdot RBC^{-1}$) was calculated by dividing ($\mu g \cdot mL^{-1}$ RBCs) by the red blood cell count. Red blood cell volume was calculated as the inverse of (red blood cell count ÷ (hematocrit/100)). Osmolarity of 100 μL of plasma diluted to 200 μL was measured by freezing point depression against diluted standards. Plasma Protein in 10–50 μL of plasma was measured by the Biuret method standardized against bovine serum albumin. Glucose in 20 μL of plasma was measured by an enzymatic technique (Sigma 1976). Liver glycogen was assayed on fresh tissue according to Hodson (1976).

Tissue Selenium

Three fish from each of the control and 50 $\mu g \cdot L^{-1}$ selenium treatments were frozen at −20°C after the 44 wk posthatch sampling. After storage for 1 yr, they were dissected still partially frozen, the tissues (Table 5) were placed in tared 100-mL volumetric flasks, weighed, and held at 4°C overnight. The subsequent selenium analysis (Agemian and Cheam 1978) was slightly modified (Agemian and Cheam 1978, unpublished data). Aqueous standards plus four subsamples each of NBS Bovine Liver 1577 (United States National Bureau of Standards) and previously analyzed feral lake trout (*Salvelinus namaycush*) whole body homogenate (archived, frozen) were included for reference. Mean tissue concentrations and concentration factors were calculated based upon measured waterborne selenium. Total body burden was calculated from the individual tissue concentrations and weights.

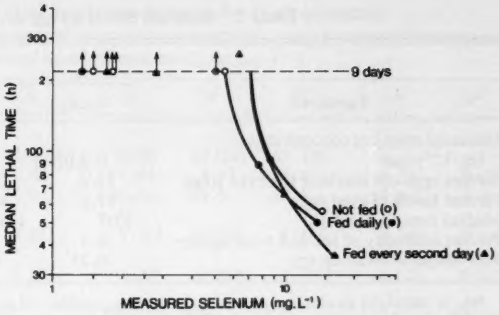

FIG. 1. Toxicity curves for acute bioassays of selenium lethality to rainbow trout. The intersection of the curves with the dashed line represents the calculated 9-d LC50s.

Results

The 9-d LC50s (with slopes and 95% fiducial limits), based on measured selenium concentrations, were 5.41 (4.42; 3.68–7.97), 6.92 (7.93; 5.33–8.97), and 7.02 (4.92; 4.99–9.89) $mg \cdot L^{-1}$ for fish that were fed daily, fed every second day or not fed at all, respectively. The mean and standard deviation of these three LC50s were 6.45 and 0.90 $mg \cdot L^{-1}$, respectively. The 96-h LC50s, interpolated from the toxicity curves (Fig. 1) were 7.2, 8.2, and 8.8 $mg \cdot L^{-1}$ for fish that were fed daily, fed every second day, or not fed at all, respectively. About 10% delayed mortality was observed in fish exposed to 1.4, 5.0, and 7.7 $mg \cdot L^{-1}$ selenium during a 4-d period following cessation of the exposure.

Selenium concentrations in water during the egg exposure were 81–94% of nominal and coefficients of variation ranged from 13 to 23% (Table 2). Selenium did not affect the survival of uneyed eggs, but eyed egg mortality increased as selenium concentrations increased and was statistically higher than control mortality at 26 and 47 $\mu g \cdot L^{-1}$. The result was a statistically significant decrease of percent hatch of eyed eggs at these concentrations although the maximum decrease from control was only 3.5% (Table 2). Observations during the eyed egg stage and at hatch indicated that the mortality occurred before, and not during hatching. Median time to hatch of exposed eggs was reduced slightly (maximum of 9%) at selenium concentrations greater than 4.4 $\mu g \cdot L^{-1}$ but the difference was statistically significant only at 16 $\mu g \cdot L^{-1}$. The increased rate of development was not detectable by staging by the method of Ballard (1973). There were no selenium related changes in wet weight at swim-up or percent mortality of sac and swim-up fry.

Overall means of selenium concentrations during the

TABLE 2. Selenium effects on egg development, hatching, and fry survival.

Parameter	Nominal selenium concentrations ($\mu g \cdot L^{-1}$)							
	0 (ctl)	5	9	16	28	50	SD[a]	$s\bar{x}$[a]
Measured selenium concentration ($\mu g \cdot L^{-1}$) (SD)	0.3 (0.1)	4.4 (1.0)	7.3 (1.2)	15 (3)	26 (4)	47 (6)	—	—
Percent eggs not reaching the eyed stage	18.4	18.1	19.4	18.1	18.1	18.6	1.8	1.0
Percent hatch of eyed eggs	97.0	94.7	95.3	94.7	94.1*[a]	93.5*	1.3	0.7
Median time to hatch (h)	1037	1057	1013	975*	993	1023	31	18
Percent mortality of sac and swim-up fry	6.4	3.9	3.6	4.4	3.9	5.0	1.8	1.0
Wet weight at swim-up (g)	0.25	0.28	0.28	0.27	0.28	0.28	0.04	0.02

[a]SD = standard deviation, $s\bar{x}$ = standard error, number of replicates = 3, * = significantly different at the 95% confidence level from control values as shown by Duncan's New Multiple Range Test.

posthatch exposure ranged from 92 to 106% of nominal but variation over 11 mo was considerable with coefficients of variation ranging from 31 to 38%. Mortality of selenium-exposed fingerlings did not differ from that of controls during the entire posthatch exposure period and appeared to be due to aggression when density decreased to less than 10 fish per tank. The physiological measurements at 23 wk posthatch (i.e. at the maximum annual temperature of 24°C) demonstrated little response of the fish to selenium exposure. Blood iron as $\mu g \cdot mL^{-1}$ RBCs increased significantly at a selenium concentration of 53 $\mu g \cdot L^{-1}$ compared to blood iron at 5.5, 8.3, and 27 $\mu g \cdot L^{-1}$, but not compared to controls (Table 3). Condition factor, plasma glucose, plasma osmolarity, plasma protein, hematocrit, red blood cell count, red blood cell volume and blood iron on a whole blood or red blood cell basis did not change in response to selenium exposure.

The physiological measurements at 44 wk posthatch, when the temperature was 7.5°C, indicated a slightly greater response to selenium than at 23 wk posthatch (Table 4). Slight, but nonsignificant decreases in plasma glucose and blood iron ($\mu g \cdot mL^{-1}$ whole blood) and increases in red blood cell counts occurred at selenium concentrations greater than or equal to 16, 8.3, and 53 $\mu g \cdot L^{-1}$, respectively. A significant decrease occurred in average red blood cell volume at 53 $\mu g \cdot L^{-1}$ selenium compared to controls. At 28 $\mu g \cdot L^{-1}$ the volume appeared lower but the difference from control was not significant.

Blood iron on a whole blood basis decreased slightly but nonsignificantly as waterborne selenium increased. Blood iron as ($\mu g \cdot mL^{-1}$ RBCs) also decreased with a significant drop at 16 $\mu g \cdot L^{-1}$ selenium. Cellular blood iron ($\mu g \cdot RBC^{-1}$) was significantly lower than control values at 16 and 53 $\mu g \cdot L^{-1}$ selenium. No changes were observed in condition factor, hematocrit, plasma protein, plasma osmolarity, and liver glycogen.

Growth was unaffected by selenium exposure and there were no interactions with source of diet. Of the three commercial diets tested, two gave identical results and the third gave the same growth rate as the others when the fish were greater than 1.0 g in weight. However, the third diet "starter" feed contained so much roughage that many swim-up fry failed to feed, and died, thereby affecting overall growth rates. These

TABLE 3. Physiological responses of rainbow trout to chronic selenium exposure for 23 wk posthatch.

Parameter	Nominal selenium concentrations ($\mu g \cdot L^{-1}$)							
	0 (ctl)	5	9	16	28	50	SD[a]	$s\bar{x}$[a]
Measured selenium concentration ($\mu g \cdot L^{-1}$) (SD)	0.4 (0.8)	5.5 (2.1)	8.3 (2.1)	16 (5)	27 (10)	53 (18)	—	—
Condition factor	1.65	1.61	1.63	1.69	1.64	1.61	0.11	0.05
Hematocrit (%)	31.7	29.7	31.8	32.6	31.3	28.9	3.1	1.2
Red blood cell count ($\times 10^6 \cdot mm^{-3}$)	1.063	1.157	1.083	1.125	1.105	1.013	0.162	0.066
Red blood cell volume ($\times 10^{-6} \mu L$)	0.292	0.271	0.300	0.295	0.293	0.306	0.047	0.019
Blood iron								
($\mu g \cdot mL^{-1}$ whole blood)	150	140	170	160	140	160	30	17
($\mu g \cdot mL^{-1}$ red blood cells)	510	450	440	500	440	610	110	45
(μg per red blood cell $\times 10^{-9}$)	140	120	140	150	110	160	40	16
Plasma glucose ($mg \cdot 100\ mL^{-1}$)	70	66	68	71	69	66	8	3.2
Plasma protein ($mg \cdot 100\ mL^{-1}$)	37	38	38	39	39	36	3	1.3
Plasma osmolarity (m Osm)	280	286	283	281	291	279	12	5

[a]SD = standard deviation, $s\bar{x}$ = standard error, number of replicates = 6.

TABLE 4. Physiological responses of rainbow trout to selenium exposure for 44 wk posthatch.

Parameter	Nominal selenium concentrations ($\mu g \cdot L^{-1}$)							
	0 (ctl)	5	9	16	28	50	SD[a]	$s\bar{x}$[a]
Measured selenium concentration ($\mu g \cdot L^{-1}$) (SD)	0.4 (0.8)	5.5 (2.1)	8.3 (2.1)	16 (5)	27 (10)	53 (18)	—	—
Condition factor	1.49	1.61	1.46	1.59	1.61	1.36	0.14	0.08
Hematocrit (percent)	29.0	28.1	24.0	32.2	23.3	27.4	4.0	2.3
Red blood cell count ($\times 10^6 \cdot mm^{-3}$)	1.058	1.022	0.881	1.114	0.911	1.254	0.204	0.117
Red blood cell volume ($\times 10^{-6}$ µL)	0.277	0.274	0.272	0.290	0.256	0.214*[a]	0.020	0.012
Blood iron								
($\mu g \cdot mL^{-1}$ whole blood)	260	240	220	220	200	220	39	23
($\mu g \cdot mL^{-1}$ red blood cells)	910	850	920	660*	860	770	87	50
(µg per red blood cell $\times 10^{-9}$)	260	230	250	190*	220	170*	27	15
Plasma glucose ($mg \cdot 100\ mL^{-1}$)	74	81	74	64	61	68	13	7.5
Plasma protein ($mg \cdot 100\ mL^{-1}$)	28	24	30	31	27	29	2.8	1.6
Plasma osmolarity (m Osm)	298	286	295	295	289	293	7	4
Liver glycogen ($mg \cdot 100\ g^{-1}$)	4800	5900	6100	4700	5200	4700	1730	1000

[a]SD = standard deviation, $s\bar{x}$ = standard error, number of replicates = 3, * = significantly different at the 95% confidence level from control values as shown by Duncan's New Multiple Range Test.

TABLE 5. Concentrations,[a] concentration factors (CF), and estimated total content of selenium in tissues of rainbow trout exposed to waterborne selenium.

Nominal exposure concentration ($\mu g \cdot L^{-1}$)	0 (Control)				50				
	Concentration ($\mu g \cdot g^{-1}$ wet weight)			Total selenium (µg)	Concentration ($\mu g \cdot g^{-1}$ wet weight)			Total selenium (µg)	
	$\bar{x}$	SD[b]	CF[b]		$\bar{x}$	SD[b]	CF[b]		R[b]
Measured waterborne selenium ($\mu g \cdot L^{-1}$)	0.4				53				132.5
Gill filament	0.34	0.02	850	0.51	0.80	0.4	15	0.91	2.35
Liver	2.42	0.54	6050	2.53	12.70	9.59	240	10.77	5.24
Intestine	0.32	0.06	800	0.27	2.99	1.51	56	1.44	9.34
Pyloric caeca	0.67	0.12	1675	3.01	3.52	2.70	66	11.32	5.25
Stomach	0.47	0.12	1175	1.05	1.68	0.54	32	2.70	3.57
Kidney	0.63	0.17	1575	0.73	1.81	0.68	34	1.64	2.87
Gonad (male)[c]	0.13		325						
Peritoneal fat[d]	0.04	0.01	100	0.06	0.16	0.12	3	0.18	4.00
Brain[e]	0.15		375	0.03	0.30		6	0.15	2.00
Opercular bone + gill arch	0.19	0.07	475	0.43	0.42	0.26	8	0.63	2.21
Carcass (muscle, bone, skin)	0.14	0.03	350	22.48	0.28	0.11	5	36.51	2.00
Whole body (estimate)	0.16			31.09	0.44			66.14	2.75
Lake trout	0.38	0.25							
NBS Bovine liver	0.96	0.04							

[a]Mean of three fish.
[b]R = ratio of treated to control tissue concentrations, CF = tissue concentration/concentration in water, SD = standard deviation.
[c]Single sample.
[d]Mean of two fish.
[e]Single pooled sample.

mortalities were not related to selenium exposure.

Selenium concentrations in the NBS Bovine Liver samples averaged 87% of the certified level of 1.1 ± 0.1 $\mu g \cdot g^{-1}$, and the coefficient of variation was 4.2%. Lake trout whole body homogenates contained a mean of 0.38 $\mu g \cdot g^{-1}$ with a coefficient of variation of 66% (Table 5).

Tissue concentrations in the control group ranged

from 0.04 to 2.42 $\mu g \cdot g^{-1}$ while those of treated fish were 2.0–9.3 times higher (R, Table 5). Tissue selenium concentrations decreased in the following order for both treatments: liver > kidney-digestive tract > gill filaments > brain-carcass-bone > peritoneal fat. The coefficient of variation in treated samples averaged 55%, about twice that of the control fish. Concentration factors (CF, Table 5) ranged from 100 to 6050 for the control fish, whereas those of the treated group were 3–240.

Estimates of whole body selenium contributed by organ showed that carcass contributed 72 and 55% in control and treated fish, respectively. For both treated and control fish, digestive tract, although second to liver in selenium concentration, contributed a higher amount, mainly from the pyloric caeca. The liver contributed the next highest amount, with kidney, gill, and peritoneal fat in order of decreasing contribution. The estimated whole body concentration for control fish was only 14% higher than the average for carcass tissue alone; in the treated group, it was 57% higher.

Discussion

This experiment demonstrated that selenium is lethal to trout at concentrations ranging from 2 to 16 $mg \cdot L^{-1}$, that fish respond physiologically to selenium concentrations above 4.3 $\mu g \cdot L^{-1}$ and that selenium residues in fish tissues increase as selenium exposure increases.

The 96-h and 9-d LC50s were between 5 and 10 $mg \cdot L^{-1}$ and were not significantly affected by feeding status during the exposure. Fed fish appeared slightly more sensitive to selenium than unfed fish but LC50s may vary by 2–5× under constant conditions (Fogels and Sprague 1977). Based on 96-h LC50s, rainbow trout are as sensitive to selenium as fathead minnows (*Pimephales promelas*) but were more sensitive than brook trout (*Salvelinus fontinalis*), channel catfish (*Ictalurus punctatus*), goldfish (*Carrassius auratus*), zebrafish (*Brachydanio rerio*), and bluegills (*Lepomis macrochirus*), (Cardwell et al. 1976; Ellis et al. 1937; Goettl et al. 1976; Niimi and LaHam 1975; Weir and Hine 1970).

Chronic responses to selenium included small increases in eyed egg mortality and development rate. The observed mortality did not represent a large number of fish but was equivalent to a doubling of control mortality at the highest selenium concentration. Increased mortality might have a detrimental impact on fish production but accelerated hatching might have the opposite effect by reducing the exposure time of eggs to other hazards.

Huckabee and Griffith (1974) exposed freshly spawned carp eggs (*Cyprinus carpio*) to 1–5 $mg \cdot L^{-1}$ selenium for 60–72 h. They noted no effects of selenium on hatchability but selenium at 1 $mg \cdot L^{-1}$ or greater greatly increased mercury inhibition of hatchability. Niimi and LaHam (1976) also found no effect of selenium (as selenium dioxide) up to 10 $mg \cdot L^{-1}$ on hatching of zebrafish embryos, perhaps due to the low permeability of the egg chorion. These results contrast with our observed effects on trout eggs, perhaps due to a species or exposure time effect.

A nonsignificant elevation of blood iron concentration was the only physiological response to selenium at a high temperature after 23 wk exposure posthatch, either because of inadequate exposure time or an ability to resist the effects of selenium imparted by acclimation to warm water. The high blood iron at 53 $\mu g \cdot L^{-1}$ selenium may have been an overcompensation for an increased oxygen requirement at high temperature.

After 44 wk exposure, and a gradual decline in temperature to 7.5°C, blood iron levels increased at all selenium concentrations. However, higher selenium concentrations reduced iron per red blood cell and red blood cell volume relative to controls suggesting an increased proportion of juvenile or new red blood cells. If this were simply a stimulation of erythropoiesis, the hematocrit would also increase. However, it did not, so that decreased cell volume may reflect accelerated mortality of mature red blood cells due to the selenium exposure. The lower iron content of red blood cells at high selenium concentration may be due to cell age or selenium interference with iron metabolism. It is unlikely that the observed level of response would be detrimental to fish in a natural situation, although a comparison of performance (growth, survival, fecundity, etc.) to physiological status in a natural situation is not available. It must also be remembered that testing at the 95% confidence level means that there is a probability of 1 false positive in 20 comparisons. Since 26 separate data sets were examined, some of the subtle responses observed may be due to random error.

Sublethal injections of selenium into catfish produced popeye, peritoneal hemorrhaging, and oedema at doses greater than or equal to 10 μg per fish (Ellis et al. 1937). The hemorrhaging was associated with a decreased red blood cell count, decreased hemoglobin concentrations, an increased white blood cell count and lymphocytosis. The decrease in hemoglobin was out of proportion to the decreased white blood cell count, suggesting a selenium effect on hemoglobin synthesis. These results are similar to those of our experiment but are more severe due to the near-lethal doses administered. Histological examination by Ellis et al. (1937) revealed extensive liver degeneration and oedema of spleen, kidney, stomach, small intestine, gill arches, and gill filaments.

Goettl et al. (1976) exposed rainbow trout for 12 mo to selenium in soft water (30–35 $mg \cdot L^{-1}$). No effect was seen on fish at concentrations of 60 $\mu g \cdot L^{-1}$ or less, but at 130 $\mu g \cdot L^{-1}$ or more, there was an increasing incidence of deformities (small, pointed heads), dark colouration over the entire body, reduced growth, skin lesions, and a bulbous anus. No histological lesions were observable in the anterior and posterior kidney, liver, spleen, heart, or brain. Gissel Nielsen and

Gissel-Neilsen (1978) exposed rainbow trout to selenium for 4 wk and observed increasing mortalities at concentrations of 400 $\mu g \cdot L^{-1}$ or greater. No mortalities occurred at 100 $\mu g \cdot L^{-1}$. These results confirm our own that no dramatic effects on fish are observable below 50 $\mu g \cdot L^{-1}$ selenium.

The residue analyses clearly demonstrated that selenium content of fish reflected selenium exposure. The high precision of the NBS Bovine Liver analyses implies high precision for the method although recovery was only 87%. The lake trout data are more variable but the variability is similar to that for the experimental samples and may have been due to contamination or nonhomogeneity. The observed value of 0.38 $\mu g \cdot g^{-1}$ was close to that of 0.49 $\mu g \cdot g^{-1}$ based on a previous analysis (M. Whittle, Great Lakes Biolimnology Lab, Canada Centre for Inland Waters, Burlington, Ont., personal communication).

Tissue selenium did not increase in proportion to the increase in selenium exposure (Table 5). This suggests that either selenium in control fish originates from their diet, or that fish regulate tissue selenium through uptake and retention at low waterborne concentrations and uptake and excretion at high waterborne concentrations. This latter mechanism is supported by higher selenium concentrations in liver, digestive tract and kidney, suggesting selenium loss through biliary and renal excretion and the observation of whole body depuration by trout with a biological half-life of 29 d (Gissel Nielsen and Gissel-Nielsen 1978). Liver and digestive tract also appear to be better indicators of selenium exposure than other tissues and whole body analyses do not clearly separate selenium-exposed from control fish. While whole body analyses showed an increase of selenium in exposed fish by close to three times control levels (R, Table 5), both concentrations are well within the range of whole body or edible portion values for fish sampled from a variety of selenium-free waters (Arthur 1972; Beal 1974; Pakkala et al. 1972; Uthe and Bligh 1971). In other words, the values found would not appear abnormal.

In summary, exposure of rainbow trout eggs to waterborne selenium resulted in a slight reduction of percent hatch of eyed eggs and slightly accelerated development rates at concentrations above 26 and 15 $\mu g \cdot L^{-1}$, or greater, respectively. Continued exposure during the juvenile stage resulted in slight hematological responses at concentrations of 16 $\mu g \cdot L^{-1}$ or greater after 44 wk exposure. Selenium residues increased in all tissues of juvenile trout, particularly in liver and the intestinal tract.

Overall, these results indicate that a water quality objective for selenium of 10 $\mu g \cdot L^{-1}$ (I.J.C. 1976) protects juvenile fish but is only marginally protective of egg development. The objective is 13 times less than chronically harmful concentrations observed by Goettl et al. (1976) and about 500–1000 times less than the lethal concentration. Since background selenium concentrations in Lake Ontario water are about 0.2 $\mu g \cdot L^{-1}$ (I.J.C. 1976), only point sources of selenium will be potentially harmful to fish.

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Effect of Individual Variability on the von Bertalanffy Growth Equation

K. J. SAINSBURY[1]

Zoology Department, University of Canterbury, New Zealand

SAINSBURY, K. J. 1980. Effect of individual variability on the von Bertalanffy growth equation. Can. J. Fish. Aquat. Sci. 37: 241–247.

The growth in length of a group of animals is examined. Each animal is assumed to grow according to the von Bertalanffy model with fixed parameters, but these parameters are allowed to differ between individuals. Equations governing the mean and variance of length at given age and growth increment at given length are provided, and their implications discussed. Results indicate that the traditional growth equation is likely to result in an underestimate of the mean value of K when either length at age or growth increment data are analyzed. This problem does not appear serious when using length at age data. However, the problems of interpretation are more serious in the case of growth increment data where serious overestimates of the reconstructed mean length at age can result. A thorough analysis of growth cannot be made for a population exhibiting individual variability in L_∞ and K from growth increment data alone. In particular a nonlinear relationship between growth increment and initial length does not necessarily imply that the von Bertalanffy model is inappropriate to the species in question. A topic urgently in need of examination is the form of the joint distribution of K and L_∞ in animal populations.

Key words: von Bertalanffy, growth models

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Nous examinons la croissance en longueur d'un groupe d'animaux. On suppose que chaque animal croît selon le modèle de von Bertalanffy avec paramètres fixes, tout en permettant à ces paramètres de différer d'un individu à l'autre. Nous donnons les équations régissant la moyenne et la variance de la longueur à un âge donné et de l'augmentation de croissance à une longueur donnée, et nous analysons leurs implications. Les résultats indiquent que l'équation de croissance traditionnelle sous-estimera probablement la valeur moyenne de K quand sont analysés soit la longueur à un âge donné, soit les données d'augmentation de croissance. Ce problème ne semble pas sérieux quand on utilise les données de longueur à un âge donné. Cependant, l'interprétation est plus difficile dans le cas de données sur l'augmentation de croissance là où peuvent résulter de sérieuses surestimations de la longueur moyenne reconstruite pour un âge donné. A partir des seules données sur l'augmentation de croissance, on ne peut faire d'analyse approfondie de la croissance chez une population montrant une variabilité individuelle de L_∞ et de K. En particulier, une relation non linéaire entre augmentation de croissance et longueur initiale ne signifie pas nécessairement que le modèle de von Bertalanffy ne convienne pas à l'espèce en question. Un sujet qui a grand besoin d'être étudié est la forme de la distribution combinée de K et de L_∞ chez les populations animales.

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THE von Bertalanffy growth equation was derived by considering the growth of an animal to be due to the difference between anabolic and catabolic processes of an animal's metabolism (von Bertalanffy 1957; Ursin 1967). Thus the familiar equations, for length l_T at age T

$$(1) \quad l_T = L_\infty(1 - e^{-KT})$$

and growth increment I during time t for initial length l_T

$$(2) \quad I = (L_\infty - l_T)(1 - e^{Kt})$$

apply to an individual animal with von Bertalanffy parameters (K,L_∞).

When considering the growth of several animals, most authors proceed without comment from the individual to the group case and fit equation 1 (e.g. Beverton and Holt 1957; Allen 1966; Tomlinson and Abramson 1961) or equation 2 (e.g. Ford 1933; Walford 1946; Fabens 1965) to data. When growth parameters vary between individuals this is not a valid progression because equations 1 and 2 do not describe the mean length at age, $E[l_T|T]$, or the mean growth increment for given initial length and "time at liberty," $E[I|l_T,t]$. The possibility that the equation describing individual growth may be different to that describing the growth of a group was raised by Chapman (1961), Southward and Chapman (1965), and Dickie (1968), while

[1]Present address: Division of Fisheries and Oceanography, CSIRO, P.O. Box 21, Cronulla 2230, Australia.

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Parker and Larkin (1959) found this to be the case for a salmonid.

This paper examines the behavior of the von Bertalanffy equation when individual variability in growth is included by considering a group of animals, each growing according to the von Bertalanffy model with its own (L_∞,K) pair. Equations analogous to 1 and 2 are developed and differences in the prediction of the two equation sets are examined.

Derivation of Equations

Consider a number of animals, each with its own pair of von Bertalanffy parameters (L_i,K_i) which it retains throughout life. Thus a frequency distribution for K or L could be formed for the whole group or for animals of a given length or age (the conditional distributions). It is assumed here that the values of L_i and K_i possessed by an animal are independent of one another, and that the conditional distributions of L and K are identical over the length or age range considered. The implications of these assumptions will be examined later.

Considering first the length at age relationship, the length l_T at age T of the ith animal with the parameter pair (L_i,K_i) is

$$l_{T,i} = L_i(1 - e^{-K_iT})$$

and for several animals randomly selected from the population, the expected length at age T is,

$$E[l_T|T] = E[L](1 - E[e^{-KT}]) \tag{3}$$

with variance

$$\begin{aligned} V[l_T|T] &= E[l_T^2|T] - E[l_T|T]^2 \\ &= E[L^2](1 - 2E[e^{-KT}] + E[e^{-2KT}]) \\ &\quad - E[L]^2(1 - 2E[e^{-KT}] + E[e^{-KT}]^2) \\ &= V[L](1 - 2E[e^{-KT}] + E[e^{-2KT}]) \\ &\quad + E[L]^2V[e^{-KT}] \end{aligned} \tag{4}$$

The growth increment I_i of the ith animal, of initial length l_i, during time interval t_i is

$$I_i = (L_i - l_i)(1 - e^{-K_it_i})$$

and so for a group

$$E[I|l,t] = (E[L] - l)(1 - E[e^{-Kt}]) \tag{5}$$

with

$$\begin{aligned} V[I|l,t] &= V[L](1 - 2E[e^{-Kt}] + E[e^{-2Kt}]) \\ &\quad + (E[L] - l)^2V[e^{-Kt}] \end{aligned} \tag{6}$$

To develop equations describing the growth of a particular group we must know how L_i and K_i vary among the members of that group. There do not appear to be any data in the literature which could provide guidance on this point. It is assumed here that L is normally distributed (mean $\bar{L}_\infty$ and variance $\sigma_{L_\infty}^2$), which is probably adequate since one would expect $\bar{L}_\infty$ to be much greater than σ_{L_∞}. However the von Bertalanffy equation is very sensitive to negative values of K, even if the probability of occurrence is very low, and assuming a normal distribution of K results in unreasonable behavior (large negative lengths). Here it assumed that K follows a gamma distribution with parameters α and β (mean $\bar{K} = \alpha/\beta$ and variance $\sigma_K^2 = \alpha/\beta^2$). This distribution is described in most statistical texts (e.g. Freund 1971) and allows considerable flexibility in the distribution of K (a family of distributions ranging from slightly skewed bell-shaped through to J-shaped distributions, which include both the exponential and chi-square distributions) but does not permit negative values.

If K follows a gamma distribution, then for a given T

$$E[e^{-KT}] = (1 + \sigma_K^2T/\bar{K})^{-\bar{K}^2/\sigma_K^2}$$

and

$$\begin{aligned} V[e^{-KT}] &= (1 + 2\sigma_K^2T/\bar{K})^{-\bar{K}^2/\sigma_K^2} \\ &\quad - (1 + \sigma_K^2T/\bar{K})^{-2\bar{K}^2/\sigma_K^2} \end{aligned}$$

Equations 3 and 4 then become

$$E[l_T|T] = \bar{L}_\infty(1 - (1 + \sigma_K^2T/\bar{K})^{-\bar{K}^2/\sigma_K^2}) \tag{7}$$

and

$$V[l_T|T] = k_1\sigma_{L_\infty}^2 + k_2\bar{L}_\infty^2 \tag{8}$$

with

$$\begin{aligned} k_1 &= 1 - 2(1 + \sigma_K^2T/\bar{K})^{-\bar{K}^2/\sigma_K^2} \\ &\quad + (1 + 2\sigma_K^2T/\bar{K})^{-\bar{K}^2/\sigma_K^2} \\ k_2 &= (1 + 2\sigma_K^2T/\bar{K})^{-\bar{K}^2/\sigma_K^2} \\ &\quad - (1 + \sigma_K^2T/\bar{K})^{-2\bar{K}^2/\sigma_K^2} \end{aligned}$$

where T may be replaced by $T' = T - T_o$ (assuming T_o is constant).

Similarly equations 5 and 6 become

$$E[I|l,t] = (\bar{L}_\infty - l)(1 - (1 + \sigma_K^2t/\bar{K})^{-\bar{K}^2/\sigma_K^2}) \tag{9}$$

and

$$V[I|l,t] = k_1\sigma_{L_\infty}^2 + k_2(\bar{L}_\infty - l)^2 \tag{10}$$

with k_1 and k_2 as for equation 8 but with T replaced by t. The value of T_o, assumed constant in the derivation, cannot be obtained from growth increment data, but can be estimated by trial and error using equations 7 and 8 if the mean length and its variance are known for a particular age-class.

Parameter Estimation

A computer program, written in Fortran IV to fit equations 9 and 10 to growth increment data is available from the author. This program provides maximum likelihood estimates of $\bar{K}$, σ_K^2, $\bar{L}_\infty$, and $\sigma_{L_\infty}^2$ assuming, as an approximation, that I is normally distributed and calculates an approximate confidence interval for each parameter. The Fletcher–Powell matrix modification method (Fletcher and Powell 1963) is used to find the maximum of the likelihood function, utilizing the subroutine provided by Kuester and Mize (1973).

Comparison with the "Classical Treatment"

In comparing equations 1 and 2 with 7 and 9, it is instructive to examine briefly the differences in behavior of $(1 - e^{-KT})$ and $(1 - (1 + \sigma_K^2T/\bar{K})^{-\bar{K}^2/\sigma_K^2})$. (The following discussion applies equally to the use of t rather than T.) For fixed $\bar{K}$, $1 - (1 + \sigma_K^2T/K)^{-\bar{K}^2/\sigma_K^2} \to 1 - e^{-\bar{K}T}$ as σ_K^2

$\to 0$, but as σ_K^2 increases $(1 - (1 + \sigma_K^2 T/\bar{K})^{-\bar{K}^2/\sigma_K^2})$ becomes progressively smaller than $(1 - e^{-\bar{K}t})$. The magnitude of this difference depends upon the actual values of $\bar{K}$ and T used and the coefficient of variation of K $(\sigma_K/\bar{K})$. Figure 1 shows the effect of $\bar{K}$ and T on the ratio $(1 - e^{-\bar{K}T})/(1 - (1 + \sigma_K^2 T/\bar{K})^{-\bar{K}^2/\sigma_K^2})$ when $\sigma_K/\bar{K} = 0.1$ and 0.3. Other values of the coefficient of variation of K provide qualitatively similar results. It can be seen from Fig. 1 that when variability of K is included in the von Bertalanffy growth model the mean length at age initially becomes progressively smaller than that predicted by the classical treatment of the model. Beyond a certain age (dependent upon the magnitude of $\bar{K}$ and the coefficient of variation of K) the difference between the two models progressively decreases with increasing age. $\sigma_K/\bar{K}$ determines the magnitude of the difference between the prediction of the two models, while the value of $\bar{K}$ determines the way in which the difference is related to age. When $\bar{K}$ is small the difference between the models becomes largest in older age-classes and persists over a wider range of age-classes than is the case for the same $\sigma_K/\bar{K}$ with a large $\bar{K}$. In terms of growth increment data, the straight line relating growth increment to initial length has a lower gradient when individual variability of K is included than is given by equation 2 with the same value of $\bar{K}$. The magnitude of this reduction depends upon the value of $\bar{K}$, $\sigma_K/\bar{K}$ and the time interval over which the growth increments were measured, but is generally small. It should be noted that these statements about equations 7 and 9 are qualitatively true for equations 3 and 5 using any distribution of K (i.e. any form of individual variability in the von Bertalanffy growth rate) since $E[e^{-K}] \geq e^{-E[K]}$.

Thus an effect of variability of K among members of a population is to decrease the average growth increment, at given l and t, and the average length, at given T, relative to the prediction made assuming all animals had the same value of K (and that that value is $\bar{K}$). Conversely, if data are analyzed using equations 1 or 2 when in fact equations 7–10 are the appropriate ones, then the estimate of $\bar{K}$ obtained will be an underestimate. Some effects of using the inappropriate form of the growth equation can be gauged by examining the conditional expectations of the two forms. In the case of growth increment data, the gradient of the growth increment vs. initial length line, $(1 - (1 + \sigma_K^2 t/\bar{K})^{-\bar{K}^2/\sigma_K^2})$, is interpreted as being equal to $(1 - e^{-\bar{K}'t})$, where $\bar{K}'$ is the apparent $\bar{K}$ obtained from equation 2. This leads to

$$(11)\quad \bar{K}' - \bar{K} = \bar{K}\left[\frac{\bar{K}}{t\sigma_K^2}\ln(1 + \sigma_K^2 t/\bar{K}) - 1\right]$$

and since $\bar{K}/t\sigma_K^2$ is positive, $\bar{K}' \leq \bar{K}$. Likewise $\bar{K}'$ obtained from length at age data is less than or equal to $\bar{K}$. When the parameters of the growth function obtained from growth increment data are used to estimate the mean length at age, $\bar{K}'$ is usually substituted into equation 1. With $\bar{l}_T'$ being the mean length at age T given by equation 1 using $\bar{K}'$, we then have

$$(12)\quad \begin{aligned}\bar{l}_T' - \bar{l}_T &= \bar{L}_\infty[(1 - e^{-\bar{K}'T}) \\ &\quad - (1 - (1 + \sigma_K^2 T/\bar{K})^{-\bar{K}^2/\sigma_K^2})] \\ &= \bar{L}_\infty[(1 + \epsilon T)^{-\frac{\bar{K}}{\epsilon}} - (1 + \epsilon t)^{-\frac{\bar{T}\bar{K}}{t\epsilon}}]\end{aligned}$$

where $\epsilon = \sigma_k^2/\bar{K}$. From this we find that $\bar{l}_t' > \bar{l}_T$ when $T > t$, $\bar{l}_T' = \bar{l}_T$ when $T = t$ and $\bar{l}_T' < \bar{l}_T$ when $T < t$. Most studies use data in which the time over which growth increments are measured is less than the age at which mean length is being estimated $(T > t)$, resulting in an overestimate of mean length at age in the reconstructed relationship. The extent of this overestimation is difficult to gauge in the absence of general knowledge on the value of the coefficient of variation of K. That serious bias can result, however, is indicated by a study of abalone (Sainsbury 1977) for which $\bar{L}_\infty = 132$ mm, $\bar{K} = 0.16$, and $\epsilon = 0.072$ with t about 0.7 yr, which results in a 13% overestimation of $\bar{l}_T$ for some age-classes when analyzed by the Fabens (1965) method. Relationships 11 and 12 do not include all possible sources of bias in the estimates of $\bar{l}_T$ or $\bar{K}$ (e.g. differences in procedures used to fit the different equation sets to data or the possibility that neither of the equation sets are appropriate), and so cannot be used to calculate $\bar{K}'$ exactly for a particular data set. However, they do provide a means of accessing the effect of a particular type of individual variability on parameter estimates made assuming that all animals grow identically.

The effect of variability in K upon the variance of length at age is shown in Fig. 2. Figure 2a provides an example of the length–age relationship when variability in K is very low $(\sigma_K/K = 0.005)$. In this case the mean length at age and its variance calculated from 7 and 8 are almost identical to those given by equations 13 and 14, and as expected from equation 14 the variance monotonically increases with age to an asymptotic value of σ_{L_∞}. The effects of increased σ_K^2 (while all other parameters remain constant) are shown in Fig. 2b. There is a slight reduction in the mean length at age, in accordance with the previous discussion, and a change in the relationship between the variance of length and age. This variance now increases with age to a maximum, and then declines for older age-classes. However, this effect is noticeable only when $\sigma_K/\bar{K}$ is quite large (as in Fig. 2b where $\sigma_K/\bar{K} = 0.35$) and could easily go unnoticed in data. When $\sigma_K/\bar{K}$ is large the variance of length for old age-classes may still contain a significant component of variation due to K, and so may be somewhat more than $\sigma_{L_\infty}^2$. It should be noted that since K appears in the original von Bertalanffy equation as KT', exactly the same effects on the length at age distributions could be obtained if K was constant and either T was determined with error or T_o varied between individuals. As both of these sources of variation will be present in most data sets their effects should be borne in mind when interpreting any analysis.

Similarly the effect of variability in K upon the variance of the growth increment at given initial length can be examined by comparing equation 10 and 16. For constant K the variance of the growth increment does not alter with the initial length considered (provided the assumptions are met — see next section). When variability in K is present the variance of the growth increment for constant time at liberty decreases with increasing initial length essentially as some fraction of $(\bar{L}_\infty - l)^2$.

These patterns of variation in length at age and growth increment may be used to gain a general idea of the level of variability of K through an examination of growth data in the literature. Many fish species show a general increase in the variance of length at age with increasing age (e.g. Grant and Spain 1975; Southward 1976; Westrheim and Ricker 1978; Steinmetz 1974; Hancock 1965), suggesting that the variance of K is not great. Exceptions to this are found in the growth increment data provided for an eel (Burnet 1969), the length at age of a myctophid (Gjosaeter

1973), and the Californian sardine (Wolf and Daugherty 1961). In a very interesting paper, Ricker (1969) also cites examples of populations of sprat and of bluegill sunfish in which the variance of length at first increases with length and then decreases, and discusses the observations in terms of growth compensation, length dependent mortality, and the relationship between growth in length and growth in weight. In molluscs a high degree of variability in K seems to be common (e.g. Feare 1970; Paul and Feder 1973; Taylor and Venn 1978; Poore 1972).

If K does not vary between individuals but L_∞ does, then the equation sets become much simplified and unbiased estimates of $\bar{K}$ and $\bar{L}_\infty$ can be obtained from equations 1 and 2, although they are still subject to the problems described in the next section. With constant K equations 3 and 4 become

$$(13)\quad E[l_T|T] = \bar{L}_\infty(1 - e^{-KT})$$

and

$$(14)\quad V[l_T|T] = \sigma_{L_\infty}{}^2(1 - e^{-KT})^2$$

while equations 5 and 6 are

$$(15)\quad E[l|l, t] = (\bar{L}_\infty - l)(1 - e^{-Kt})$$

$$(16)\quad V[l|l, t] = \sigma_{L_\infty}{}^2(1 - e^{-Kt})$$

Variability in L_∞ does not influence the mean growth increment or mean length at age of a group because L_∞ occurs as a linear term in the original von Bertalanffy equation.

Some Problems of Analysis and Interpretation That Arise from Individual Variation

There is a very basic difference between the construction of an age–length relationship from age data and from growth increment data. When age data are analyzed it is unlikely that there will be any major differences in interpretation of the age–length relationship when using either version of the von Bertalanffy equation (1 or 7). This is because the regression line is generally used as a summary of the data, and interpretation in terms of the growth model is not required. The major effect of individual variability in this case is that the value of $\bar{K}$ obtained is an underestimate of the actual mean K, but for biologically reasonable parameter values this bias is quite small (see Fig. 1). However, there can be a considerable difference between the two treatments of the von Bertalanffy model when the age–length relationship is constructed from the analysis of growth increment data (equations 2 and 9). As illustrated by equation 12, the predictions made assuming no variability in the growth parameters may be considerably larger than those made assuming that the parameters do vary between individuals. The difference between treatment arises not so much from a difference in the estimated mean K and L_∞, but because for growth increment data the estimated parameter values must be used in conjunction with the underlying growth model in the construction of an age–length relationship. And so use of the correct model (which need not be the one described here) becomes extremely important.

A problem of analysis highlighted by the examination of individual variability is that most methods of fitting the von Bertalanffy equation (e.g. Fabens 1965; Allen 1966) do not permit the variance of the dependent variable, length or growth increment, to alter with the independent variable, age, or initial length, respectively. Equations 8 and 10 show that these variances are a function of the independent variable whenever there is individual variability in K or L_∞. The effects of this upon analysis could be many and varied, and stem from too much weight being given to observations of the dependent variable in the region where its variance is high. Thus the analysis will be overly sensitive to chance events or bias affecting observations in this region of the independent variable.

An important problem to emerge from consideration of individual variability in growth parameters concerns the effects of changes in the conditional distributions of K and L_∞ (conditioning is on age or initial length). That the conditional distributions do not alter is a key assumption in the derivation of the equations provided, and any violation will result in departures from them. (It should be noted that departures from the traditional form of the equations will also occur.)

In the case of length at age data, sampling bias, and mortality rates that depend upon growth rate and/or size are the most likely cause of age specific distributions of growth parameters. Although it is often difficult to overcome these problems, they have long been recognized as having an influence on growth parameter estimates and have been cited as possible causes of the well known Lee's phenomenon (see Ricker 1975, p. 125). In the case of growth increment analysis, however, we have a far more difficult problem and one which does not seem to be generally recognized. Besides the possibility of a mortality rate or sampling probability which may be a function of the K_i, L_i) pair of an individual, there are two other factors which will alter the conditional (upon initial length) distributions of K and L_∞. These are (i) the age composition of the sampled group and (ii) truncation of the L_∞ distribution when large animals are considered.

(i) The age composition — As a cohort ages it will be sorted in terms of length so that the mean values of K and/or L_∞ for large animals will be greater than those for small animals. A plot of growth increment against initial based on animals tagged from such a cohort would have a less negative slope than the cohort mean K and L_∞ would suggest. (See the data presented by Hancock 1965.) When more than one cohort is present in the tagged population the overall effect of this sorting is to introduce nonlinearity into the relationship between the growth increment and initial length. The severity of this problem is dependent upon the age composition of the tagged group, whether and to what extent K_i and L_i are correlated for individuals, and the coefficient of variation of K and L_∞. When there is large individual variability in the growth parameters a

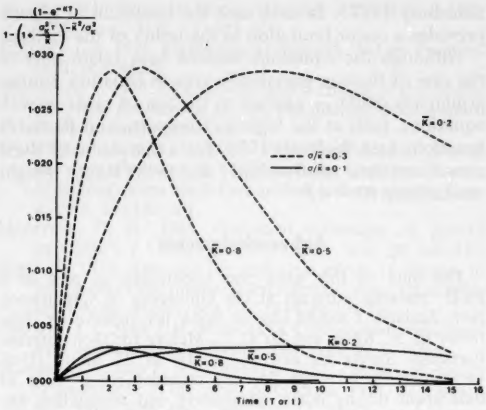

FIG. 1. The effect of variability in K on the relative difference between the traditional and the present growth equations. The difference increases as $\sigma_K/\bar{K}$ increases, and is most pronounced in more, older age-classes as $\bar{K}$ decreases (or in longer times at liberty for the case of growth increment data).

domed relationship between the growth increment and initial length can arise, the exact form of which is influenced by the age composition (perhaps the case in the data presented by Frank 1965). From this it is concluded that a thorough analysis of growth can be obtained only when the age composition can be determined. Any estimates based upon growth increment data alone must be regarded as approximations subject to sources of variation over which the researcher may have no control and very little knowledge. In some populations (e.g. those with low levels of individual variation of growth parameters and an unchanging age composition) the problems may be minor, but in others the potential for error and confusion is great. It would appear that many molluscs fall into the latter category.

(ii) Truncation of the L_∞ distribution — If L_i is fixed for an individual then clearly L_i must be greater than or equal to that animal's length at tagging. Thus when considering the growth of individuals whose initial length is within the range of L_∞ values for the population as a whole, only part of that distribution is relevant. This successive truncation of the L_∞ distribution as larger animals are considered will cause the relationship between growth increment and initial length to become curvilinear when the initial length considered is larger than the L_∞ value of some animals in the sample (i.e. encroaches the domain of the L_∞ distribution, see Fig. 3). This phenomenon is to be expected whenever there is individual variability in asymptotic length. It is evident in many sets of published data (e.g. Frank 1969; Poore 1972; Ricker 1975, p. 223; Manzer and Taylor 1947; Dipper et al. 1977) and will tend to cause an overestimate of $\bar{L}_\infty$ and an underestimate of $\bar{K}$.

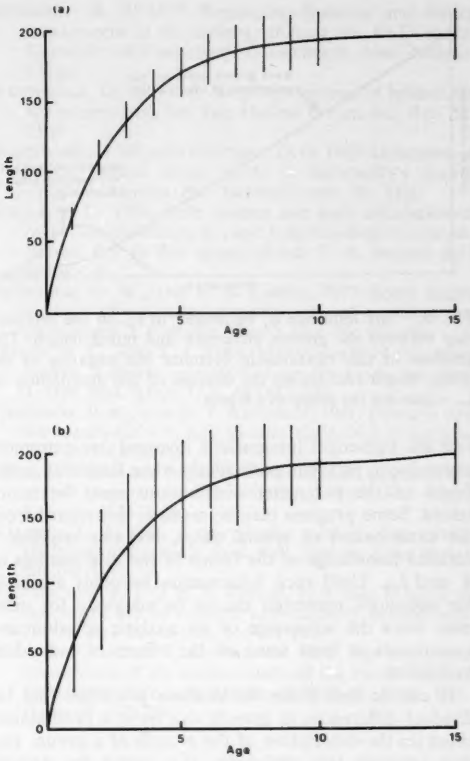

FIG. 2. The effects of variability in K upon the mean and variance of length at given age. In each case $\bar{L}_\infty = 200$, $\sigma_{L_\infty}^2 = 100$, $\bar{K} = 0.4$ and the vertical bars represent $\pm 2\,\sigma_{l_T}$. (a) $\sigma_K/\bar{K} = 0.005$. This is almost indistinguishable from the case of $\sigma_K^2 = 0$, and shows a monotonic increase in the variance of length with age. (b) $\sigma_K/\bar{K} = 0.35$. The mean length at age is slightly less than that shown in (a), and the variance of length at first increases, and then decreases with increasing age.

Discussion

The equations developed here should be regarded as a "first analysis" of the effects of individual variation on the description of growth for a group. The equation sets are more realistic than previous approaches, but many difficulties remain. Some of the problems (notably determination of the exact distribution of $l_T|T$ and $l|l_T$, t and the effects of varying conditional distributions of K and L_∞) can be overcome by developing the equation governing the joint probability density function $f(l_T, l_{T+t}, T)$ in terms of the distributions of K, L_∞ and T. This is clearly the correct way of approaching the problem, rather than an estimation of just the first two moments as has been presented here. However, there is no analytic solution for the joint density function

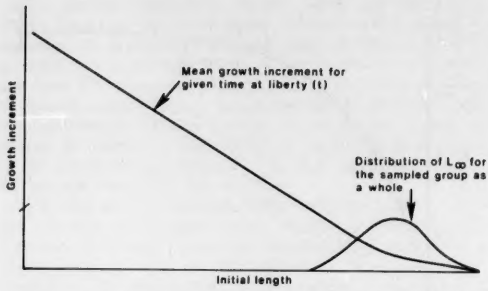
Growth increment
Mean growth increment for given time at liberty (t)
Distribution of L_∞ for the sampled group as a whole
Initial length

FIG. 3. The influence of variability in L_∞ on the relationship between the growth increment and initial length. The gradient of this relationship becomes less negative as the initial length encroaches the domain of the distribution of L_∞ values for the group as a whole.

and the numerical integrations involved are extremely expensive to perform, particularly when flexibility in the forms of the parameter distributions must be maintained. Some progress may be made in this regard from the examination of special cases, but this requires a detailed knowledge of the forms of the distributions of K and L_∞. Until such information becomes available the equations presented should be adequate for most uses, have the advantage of an analytic solution and incorporate at least some of the effects of individual variability.

It can be seen from the analysis presented that individual differences in growth can have a pronounced effect on the description of the growth of a group, and that ignoring this variability (i.e. using the growth equation for an individual to describe the growth of a group) can result in biased estimates of the mean parameter values. It is clear that the usual treatment of growth is too simplistic, and that a curvilinear relationship between growth increment and initial length does not necessarily indicate that the von Bertalanffy model is inadequate for the species under consideration.

The effect of individual variability is particularly important when interpreting growth increment data, and can result in serious overestimates of the mean length at age of a cohort. This could have a major influence on quantities such as yield estimates (Beverton and Holt 1957; Jones 1974; Southwood and Chapman 1965) and mortality estimates (Beverton and Holt 1958; Ebert 1973) derived using a growth curve fitted to tag return or other growth increment data. A more complete description of the growth process, including the proper incorporation of individual variability, will allow the development of more reliable methods of analysing populations based on size rather than age data. This approach has obvious advantage in that size data are generally easier to obtain than age data, and several attempts have already been made (e.g. Baranov 1918, in Ricker 1975, p. 60; Beverton and Holt 1956; Ebert 1973; Jones 1974; Ssentongo and Larkin 1973; Sainsbury 1977). In each case the treatment of growth provides a major limitation to the utility of the method.

Although the equations derived here relate only to the case of the von Bertalanffy growth equation, similar arguments could be applied to the use of other growth equations, such as the logistic, Compertz and Richards functions (see Richards 1959, for a discussion of these curves and their relationships) and to the length–weight relationship $W = a\,l^b$.

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Influence of Secondarily Treated Kraft Mill Effluent on the Accumulation Rate of Attached Algae in Experimental Continuous-Flow Troughs

MAX L. BOTHWELL[1]

Environmental Technology Department, Corporate Research Division, Weyerhaeuser Co., Tacoma, WA 98401, USA

AND JOHN G. STOCKNER

Department of Fisheries and Oceans, Fisheries Management — Pacific, West Vancouver Laboratory, West Vancouver, B.C. V7V 1N6

BOTHWELL, M. L., AND J. G. STOCKNER. 1980. Influence of secondarily treated kraft mill effluent on the accumulation rate of attached algae in experimental continuous-flow troughs. Can. J. Fish. Aquat. Sci. 37: 248–254.

In three seasonal experiments in 1977, increases in the rate of attached algal accumulation were normally found with each additional increment of secondarily treated kraft mill effluent (KME). The effect of KME on the instantaneous accumulation coefficients of algae were 1.2–2.8 times greater in the summer than in the autumn or spring at all dilutions tested. At higher concentrations (5 and 25% v/v KME) an inhibition of accumulation was usually observed during the first 5–6 d of exposure; after this period diatom biomass increased sharply. Succession of diatom taxa to different forms, rather than physiological adaptation by usually predominant species occurred. No inhibition was noted at lower concentrations (0.5, 1, and 2% v/v) of KME. Relatively high levels (20–30 $\mu g \cdot L^{-1}$) of soluble reactive phosphorus (SRP) and low concentrations of nitrate + nitrite-nitrogen (5–10 $\mu g \cdot L^{-1}$) and ammonia-nitrogen (2–20 $\mu g \cdot L^{-1}$) in the river dilution water suggested that nitrogen was the primary limiting nutrient. Ammonium uptake tests for nitrogen deficiency confirmed nitrogen limitation in algae grown in unenriched river water compared to algae exposed to KME for 6 d. Differences in chlorophyll *a*: ATP ratios among periphyton communities of the different treatments were minimal after 9 d.

Key words: kraft mill effluent, algal growth, nutrient enrichment, benthic algae, river, chlorophyll *a*

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Au cours de trois espériences siasonnières en 1977, on a observé des augmentations du taux d'accumulation d'algues fixées avec chaque augmentation supplémentaire d'effluent d'une fabrique de papier kraft (KME) soumis à un traitement secondaire. L'effet du KME sur les coefficients d'accumulation instantanés des algues est de 1,2 à 2,8 fois plus élevé en été qu'en automne ou au printemps à toutes les dilutions essayées. Aux concentrations élevées (5 et 25% v/v de KME) il y a ordinairement restriction à l'accumulation durant les premiers 5–6 jours d'exposition; après cette période, la biomasse des diatomées augmente de façon abrupte. Il y a succession de taxons de diatomées à différentes formes, plutôt qu'adaptation physiologique des espèces ordinairement prédominantes. Aux basses concentrations (0,5, 1 et 2% v/v) de KME, nous n'avons pas observé de restriction. Les niveaux relativement élevés (20–30 $\mu g \cdot L^{-1}$) de phosphore réactif soluble (SRP) et les faibles concentrations de nitrates + azote nitrique (5–10 $\mu g \cdot L^{-1}$) et d'azote ammoniacal (2–20 $\mu g \cdot L^{-1}$) dans l'eau de dilution de la rivière donnent à croire que l'azote est l'élément nutritif limitatif principal. Des essais d'absorption d'ammoniac en vue de déterminer une déficience d'azote confirment l'effet limitatif de l'azote chez des algues cultivées dans de l'eau de rivière non enrichie, comparativement aux algues exposées au KME pendant 6 jours. Après 9 jours, les différences de rapports chlorophylle *a*: ATP chez les communautés périphytoniques soumises aux divers traitements sont minimales.

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[1]Present address: Department of the Environment, National Water Research Institute, Pacific and Yukon Branch, 4160 Marine Drive, West Vancouver, B.C. V7V 1N6.

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THE potential eutrophying effect of nutrients in kraft mill effluent (KME) has received little attention. This is primarily because other properties of KME such as toxicity, oxygen demand, and light attenuating color often have more environmental significance (Walden 1976; Parker and Sibert 1973; Parker et al. 1975; Stockner and Cliff 1976). The first two of these are known to be removed or substantially reduced by secondary biological oxidation (Mueller and Walden 1974; Rainville et al. 1975). Consequently studies identifying phytotoxicity of untreated KME have little relevance to the potential impact of oxidized KME on algal production in situ (Moore and Love 1977; Eloranta 1978). A reduction in toxicity should allow effects of nutrient addition by treated KME to be observed. While this has been documented in batch culture type experiments (Stockner and Costella 1978), light reduction by effluent color in deeper waters usually masks any eutrophication effect (Parker and Sibert 1973; Stockner and Cliff 1976). Attached algae in shallow flowing water would seem most likely to show a response to nutrient stimulation by secondarily treated KME. This paper reports the results of three such experiments conducted on riverine diatoms in continuous-flow troughs.

Materials and Methods

Six plexiglass troughs (3.6 m × 19 cm) used in the experiments were located on the bank of the McKenzie River, Oregon. River water was pumped continuously to a constant head tank and distributed to each of six 20-L mixing chambers. Fresh secondarily treated kraft mill effluent (KME) was pumped continuously into a head tank on the site and dispensed into five of the six mixing chambers. Various combinations of effluent and river water from the mixing tanks were fed to the head of the troughs by PVC pipes. Flow velocity in each trough was 50 cm·s^{-1}. Water depth was 0.8 cm and total discharge from each trough was 46 L·min^{-1}. The KME dilutions used in all experiments were 0.5, 1, 2, 5, and 25% v/v. The sixth trough served as the control and received only river water.

The hydraulic residence time in the complete system was less than 30 s, assuring that the water quality in the control trough was identical at all times to that of the river. Flushing time for the troughs alone of only 7 s prevented any measurable alteration in soluble nutrients from the head to the foot of the troughs.

Experiments were conducted during three different seasons in 1977 to test the effect of KME under various light and temperature regimes. While some details of the trough apparatus and sampling procedures varied between trials, the same experimental design was used in all. Measurements of chlorophyll *a* density were made at frequent intervals during the period of exponential increase of algal biomass. Experiments were terminated when sloughing became evident. Chlorophyll *a* data were fitted to an exponential growth equation:

$$y = ae^{tk}$$

Where: y = chlorophyll *a* density (mg·m^{-2}) at time, t
a = initial chlorophyll density (mg·m^{-2})
t = time in days
k = instantaneous accumulation coefficient (d^{-1})

The instantaneous accumulation coefficients for the treatments in each experiment were calculated using only the chlorophyll data from either day 5 or 6 until sloughing started. This helped minimize the effect of initial colonization on the calculation of k and had the additional advantage of eliminating interference from an initial inhibitory phase at higher KME levels. Macroinvertebrates colonizing the troughs were removed with forceps on each sampling date to reduce grazing effects. Under conditions of identical substrate, flow velocity, light and temperature, differences in the rate of algal growth as a function of nutrient stimulation by KME were reflected by differences in the accumulation rate of chlorophyll. The approach was similar to the method of Kevern et al. (1966), which provides a reliable estimate of net primary production.

Water temperatures in the troughs were measured daily with an immersion thermometer. Solar irradiance was monitored with an integrating Eppley pyrohcliometer and mean daily values were calculated for each experimental period.

Water samples taken from the ends of the troughs were filtered, preserved with mercuric chloride, and analyzed for soluble reactive phosphorus (SRP), nitrate + nitrite–nitrogen, dissolved organic nitrogen (DON) and ammonium–nitrogen by the methods of Strickland and Parsons (1972). Samples for these analyses were collected every 2–3 d during the first two experiments and daily during the third trial.

Experiment 1 Spring (March 30–April 25, 1977)

Glass microscope slides positioned horizontally in the troughs served as colonization substrates. Each trough was divided into two light regimes: full sunlight and a 50% reduction using neutral density screens. Samples for chlorophyll *a* analysis were taken 2, 6, 9, 16, 20, and 26 d after start up. Two slides were removed from each treatment and extracted directly in 90% acetone. Pigment extraction, aided by 2 min of ultrasonic disruption, continued overnight at 5°C in the dark. Chlorophyll *a* was determined by either the fluorometric method of Holm-Hansen et al. (1965) or, at higher concentrations, the spectrophotometric technique of Lorenzen (1967). Both methods corrected for the presence of pheopigments.

Experiment 2 Summer (August 11–22, 1977)

In the second and third experiments, continuous sheets of styrofoam-DB (0.6 cm thick) replaced glass slides as the substrate. The use of styrofoam eliminated the problem of the "leading edge" pattern of algal accumulation noted on the glass slides.

Glass plates (1 cm thick) covered the troughs to attenuate UV-radiation which in the first trial inhibited microbial colonization. Additionally, this precaution prevented rain from dislodging attached algae and kept debris out of the troughs.

Chlorophyll *a* samples were taken 3, 5, 8, and 11 d after initiation by removing 4.21 cm^2 cores of the foam with a cork borer. Six replicate cores from each treatment were frozen and later ground in 90% acetone with a tissue grinder. Depending upon the amount of pigment present,

either fluorometric or spectrophotometric analysis was used. Preliminary tests established that the styrofoam which dissolved in the acetone extraction procedure did not interfere with either method of chlorophyll determination.

EXPERIMENT 3 AUTUMN (OCTOBER 18–28, 1977)

With the exception of day 7, samples were taken daily during the third experiment. Four replicate styrofoam cores from each trough were taken for chlorophyll determination.

Additionally, daily ATP measurements quantified the total living periphyton biomass in each treatment. Material from 1 to 4 cores was washed into a 100-mL graduated mixing cylinder by several short bursts of sterile filtered river water. After vigorous agitation to disrupt clumps of diatoms and bacteria, 1 mL of the cell suspension was injected directy into 15 mL of boiling sterile 0.02 M Tris buffer, pH 7.7. The extraction continued for 2–3 min after which the extract was carefully decanted into sterile vials, sealed and frozen. Subsequent ATP analysis followed the method of Jones and Simon (1977).

Algal material from each trough was collected daily and fixed with Lugol's acetate for taxonomic identification. Permanent mounts of the nitric acid-cleaned diatom frustules were used to identify and estimate the relative abundance of taxa.

Physiological tests for nitrogen and phosphorus deficiency were made after 6 d of exposure to the various concentrations of KME. On day 7 algae from each trough were transported on ice to the laboratory and thoroughly washed in nitrogen and phosphorus-free Guillard's Woods Hole MBL medium (Nichols 1973). Duplicate aliquots of the resuspended diatoms were then used for ammonium uptake determinations following the procedure of Fitzgerald (1968), and boiling buffer extractable phosphorus measurements and alkaline phosphatase activity determinations using the methods of Fitzgerald and Nelson (1966).

Results

Mean daily solar insolation values and river temperature ranges during the three experimental periods were 1635 $J \cdot cm^{-2} \cdot d^{-1}$, 8–10°C; 1915 $J \cdot cm^{-2} \cdot d^{-1}$, 15–18°C and 648 $J \cdot cm^{-2} \cdot d^{-1}$, 10–12°C for the spring, summer, and autumn, respectively. In the spring trial, the light exposure received by the 50% shaded sections of the troughs was 818 $J \cdot cm^{-2} \cdot d^{-1}$.

The KME temperature normally ranged from 25 to 30°C; consequently, elevated temperatures accompanied the higher KME treatments. At KME concentrations of 0.5% through 2% the temperature increases were usually undetectable and always less than one degree. At 5% KME the increase was 1–1.5°C while 25% KME resulted in an elevation of 5–6°C.

Diatoms were the predominant algal colonizers throughout each experiment. Toward the end of all three experiments much greater levels of chlorophyll *a* had accumulated at 5 and 25% KME than at lower concentrations, and higher amounts of accumulation usually accompanied each additional increment of KME above the control (Table 1). Some exceptions occurred in the first experiment when sloughing took place during the final 10 d. This was most severe in the 25% KME treatment and sampling of that trough was discontinued after day 16. Sloughing also apparently occurred in the 2% KME treatment resulting in lower chlorophyll values on day 26 than on day 20. In addition, inhibition of microbial accumulation by UV-radiation was found in those sections of the troughs exposed to unattenuated sunlight. Chlorophyll levels in those sections were 2–10 times lower than in shaded areas of the same trough. Consequently only results from the shaded treatments were considered valid for assessing KME effects and for comparison with accumulation data in the two subsequent experiments.

TABLE 1. Chlorophyll *a* ($mg \cdot m^{-2}$) accumulated on artificial substrates during three seasonal experiments.

Day	Percent (v/v) kraft mill effluent					
	Control	0.5%	1%	2%	5%	25%
	Spring experiment[a] *Mar. 30, 1977–Apr. 25, 1977*					
2	0.3	0.3	0.3	0.2	0.4	0.06
6	1.5	1.1	1.0	1.7	1.0	0.2
9	1.1	3.3	3.8	6.0	5.1	2.3
16	3.0	3.3	9.9	13.3	18.3	29.5
20	8.6	30.3	27.8	46.9	100.0	NS[c]
26	40.4	65.2	64.8	21.3	386.6	NS[c]
	Summer experiment[b] *Aug. 11, 1977–Aug. 22, 1977*					
3	5.7	4.4	4.6	3.4	4.8	2.0
5	14.5	11.4	12.0	9.0	10.2	6.4
8	16.8	30.5	37.0	35.2	81.0	64.4
11	50.6	115.2	171.2	178.2	246.4	255.2
	Autumn experiment[b] *Oct. 18, 1977–Oct. 28, 1977*					
1	1.8	1.9	2.2	1.8	0.8	0.4
2	5.3	6.6	7.2	5.2	2.2	0.8
3	10.5	10.0	13.0	8.6	4.7	2.9
4	15.7	15.1	17.5	13.6	8.1	4.6
5	18.5	20.8	24.8	23.8	15.0	6.8
6	22.1	26.0	34.1	34.6	24.0	18.0
8	31.6	34.0	46.8	54.7	81.0	46.2
9	42.1	42.1	55.2	56.8	81.0	63.6
10	38.4	41.6	56.9	63.1	122.0	87.3

[a]Glass slide substrate.
[b]Styrofoam — DB substrate.
[c]Not sampled due to obvious sloughing.

The stimulatory influence of KME on algal accumulation was clearly shown in the first experiment and was subsequently corroborated in the summer and autumn trials (Table 1). In the summer trial, chlorophyll at 5% and 25% KME was significantly higher ($P < 0.05$) than either the control or the lower KME levels on both the eighth and eleventh days, with values on the final day being about 5 times greater than the control (Table 1). Even the 1 and 2% KME treatments were significantly higher ($P < 0.05$) than the control or the 0.5% KME treatment on day 11. Simi-

larly, on the final day of the autumn experiment the two troughs receiving the highest amounts of KME had significantly greater ($P < 0.05$) chlorophyll density than the control or the lower level KME treated troughs (Table 1). Chlorophyll values in the 1 and 2% KME treatments were also greater ($P < 0.05$) than either the control or the 0.5% KME treatment at the end of the autumn experiment.

During the first 5–6 d of each experiment chlorophyll levels in troughs exposed to 25% KME were substantially lower than the controls or the 0.5 and 1% KME treatments (Table 1). This effect was most notable in the autumn trial when even the 5% KME treatment exhibited significantly less ($P < 0.05$) accumulation than lower concentrations of KME during the first 4 d (Table 1). The depression of diatom accumulation by concentrations of KME 5% or greater was clearly reversed after approximately 5–6 d of exposure when rapid growth ensued. Taxonomic analysis of the diatom communities during the third experiment revealed that succession to different taxa had occurred at the 5 and 25% KME concentrations rather than physiological adaptation by the initial colonizers. In those two troughs *Diatoma hiemale*, *Synedra ulna*, *Fragilaria vaucheriae*, and two species of *Nitzschia* emerged as exclusive predominants after 3–6 d. These species were either absent or very sparse in the control and at KME concentrations up to 2%. There was no such successional trend in the lower treatment levels where the diatom communities were comprised of about 25 taxa (Table 2) with no clear predominant species.

The capacity of KME to increase the rate of algal accumulation, i.e. growth, was observed during all three experiments (Table 3). However, the stimulatory effect of concentrations up to 1% was minimal during the autumn and spring trials. Concentrations of 2% KME elevated k values 29 and 19% above those of controls in the spring and autumn, respectively. At 5% KME or greater the algal response was substantial, with approximately a twofold increase in k observed at 25% KME during the autumn and spring. The greatest response to KME occurred in summer when river water temperature was 5–7°C warmer and solar irradiance in the troughs was more than twice spring and autumn levels. During summer, k in the control was also higher than in the controls of the two other experiments. Exposure to only 0.5% KME resulted in a doubling of algal accumulation rate and 25% KME nearly tripled values of k (Table 3).

Concentrations of the major soluble plant nutrients in the three experimental periods with values of 20–30 $\mu g \cdot L^{-1}$ SRP, 5–10 $\mu g \cdot L^{-1}$ $NO_3^- + NO_2$ -N, 2–20 $\mu g \cdot L^{-1}$ NH_4^+ -N and 45–150 $\mu g \cdot L^{-1}$ DON. At higher effluent dilutions nutrient concentrations were usually not elevated measurably above river levels. This was most notable in the spring and summer experiments when no analytically detectable increases were found for SRP or $NO_3^- + NO_2^-$ -N concentrations in those troughs receiving up to 5% KME. Even at 25% KME, SRP, and $NO_3^- + NO_2^-$ -N levels were raised by only 10 and 15 $\mu g \cdot L^{-1}$, respectively. Ammonia and DON values in the 0.5 and 1% KME troughs during the first two experiments also were not measurably elevated above the control. At 5% KME, however, concentrations of these two nitrogen sources were approximately double those of unaltered river water.

TABLE 2. Diatoms identified from control trough and 1, 5, and 25% KME experimental troughs during autumn experiment. (* Predominant in 5 and 25% KME after 5–6 d in autumn experiment.)

Centrales
- *Cyclotella ocellata* Pant.
- *Stephanodiscus astrea* var. *minutula* (Kutz.) Grun.

Pennale

Araphidineae
- **Fragilaria vaucheriae* (Kutz.) Peters
- *Fragilaria construens* Ehr. Grun. var. *binodis*
- *Fragilaria brevistriata* Grun.
- **Diatoma hiemale* (Roth) Heib.
- *Diatoma vulgare* Bory.
- **Synedra ulna* (Nitz.) Ehr. var. *ulna*
- **Synedra ulna* (Nitz.) Ehr. var. *ramesi*
- *Hannaea arcus* (Ehr.) Patr. var. *arcus*
- *Asterionella formosa* Hass.

Biraphidineae
- *Epithemia argus* (Kutz.)
- *Cymbella turgida* (Greg.) Cl.
- *Cymbella cistula* (Ehr.) Kirchn.
- *Cymbella cf. tumidula* Grun.
- *Cymbella minuta* Hilse ex. Rabh.
- *Gomphonema truncatum* Ehr.
- *Gomphonema clevei* Fricke
- **Nitzschia frustulum* (Kutz.) Grun.
- **Nitzschia laueneburgiana* Hust.
- *Rhoicosphenia curvata* (Kutz.) Grun.

Monoraphidineae
- *Achnanthes minutissima* Kutz.
- *Achnanthes lanceolata* (Breb.) Grun.
- *Cocconeis placentula* Ehr.

TABLE 3. Instantaneous accumulation coefficients (k) calculated for each experimental period.

Treatment	Spring Day 6–26	Summer Day 5–11	Autumn Day 5–10
Control	0.17	0.21	0.16
0.5% KME	0.20	0.39	0.14
1% KME	0.20	0.44	0.17
2% KME	0.22[a]	0.50	0.19
5% KME	0.29	0.53	0.43
25% KME	0.48[b]	0.61	0.49

[a] k calculated with data for day 26 omitted because of sloughing loss.

[b] k calculated with data for day 20 and 26 omitted because of sloughing loss.

TABLE 4. Nutrient deficiency tests run on day 7 of the autumn experiment. Values given are means of duplicate analyses.

Treatment	Ammonium uptake rate $\mu g\ NH_4^+N \cdot \mu g\ Chl\ a^{-1} \cdot h^{-1}$	Boiling buffer extractable — P $\mu g\ P \cdot mg\ dry\ wt \cdot^{-1}$	Alkaline phosphatase activity O.D. units (395 nm)· $mg\ Chl\ a^{-1} \cdot h^{-1}$
Control	0.20	0.39	1.81
0.5% KME	0.25	0.43	2.02
1% KME	0.14	0.94	1.76
2% KME	0.12	1.33	1.37
5% KME	0.10	1.12	1.68
25% KME	0.07	—	3.48

TABLE 5. Chlorophyll *a*: ATP ratio in troughs during the autumn experiment.

Day	Percent (v/v) kraft mill effluent					
	Control	0.5%	1%	2%	5%	25%
1	8.4	5.3	4.8	6.0	1.1	0.28
2	7.9	8.3	6.2	4.8	1.6	0.44
3	6.6	7.0	7.4	6.1	3.5	1.5
4	5.4	6.3	4.0	4.5	2.9	1.1
5	6.2	7.5	9.5	7.9	4.2	0.9
6	5.1	4.4	4.6	5.3	4.0	3.0
8	5.8	5.9	6.3	4.7	6.4	2.5
9	6.9	4.7	5.6	4.8	5.1	3.9

In the autumn trial effluent nutrient levels were 5–10 times higher and more erratic than observed in the earlier experiments. As a result SRP was raised significantly ($10\ \mu g \cdot L^{-1}$) starting at the 1–2% KME level, while ammonia was increased by almost 50% at the highest dilution.

Nutrient deficiency tests during the autumn experiment provided strong evidence that the KME was supplying additional nitrogen to the attached communities in the troughs. A clear trend of decreasing ammonium uptake rates with higher KME levels was evident (Table 4). The rate of ammonium uptake per unit chlorophyll *a* in the 25% KME treatment was only a third of the value for the control. Since uptake rates of ammonium are higher in N-starved algae than in N-sufficient cells (Syrett 1962), these data suggest that diatoms in the control trough were more deficient in nitrogen than those in troughs receiving effluent.

Of the two tests used for estimating phosphorus deficiency, one indicated that the KME was also supplying additional phosphorus for algal growth. The boiling Tris buffer procedure extracted three times more phosphorus from periphyton communities exposed to 2 and 5% KME than from the control. A general trend toward increased extractable phosphorus with increasing KME concentrations was also observed (Table 4). However, these results were not corroborated by alkaline phosphatase determinations. We anticipated that higher alkaline phosphatase activity would accompany the lower intracellular P levels in the control trough. This did not occur and no well defined influence of KME on the activity of phosphatase was seen (Table 4). A possible explanation for this apparent inconsistency is that the algae had been recently exposed to a large pulse of phosphorus. Under such conditions the cells could rapidly increase their P content in a time frame too short to allow depletion of phosphatase activity per cell by division (Fitzgerald 1969). This interpretation suggests that the effluent was only sporadically supplying phosphorus.

By far the highest phosphatase level was noted in the 25% KME treatment. This treatment had a substantially larger bacterial component as shown by the low chlorophyll *a*:ATP ratio (Table 5). Bacterial phosphatases may well have interfered with analyses of samples from this trough resulting in falsely elevated estimates of algal phosphatase.

Chlorophyll *a*:ATP ratios of unaltered river periphyton communities were between 5.1 and 8.0. Values below 2 were assumed to be communities whose biomass was dominated by heterotrophs. It was clear from ratios as low as 0.28 that early colonization of the styrofoam in those troughs receiving 5 and 25% KME was largely bacterial (Table 5). This result was expected for two reasons. 1) Bacteria in the effluent initially adhered strongly to the rough textured styrofoam surface, and 2) algal colonization and/or early growth was apparently inhibited by higher KME concentrations during the first 5–6 d. At the end of 9 d, however, following exponential algal growth the relative abundance of algae and heterotrophs differed only marginally among treatments (Table 5).

Discussion

In all three experiments KME stimulated the rate of algal accumulation, i.e. growth, the effect being greater at higher concentrations. The highest levels of KME caused an initial depression in diatom colonization followed by rapid exponential growth. The similar trends seen in each experiment suggested that differences in technique between the trials had no appreciable influence on the outcome. In particular, the similarity of results using either glass or styrofoam was striking. Although styrofoam colonized much more rapidly than glass, accumulation coefficients calculated after initial colonization were roughly equivalent for the spring and autumn trials when light and temperature exposure in the troughs were of the same order. Similarities between experiments also demonstrated that cholorophyll accumulation was a consistent index of relative algal growth rates.

At KME concentrations greater than 2% heat addition by the effluent as well as nutrient stimulation would have influenced algal growth rate. Assuming a

Q_{10} value of 1.88–2.08 for algal growth (Epply 1972; Goldman and Carpenter 1974), the 1–1.5°C increase observed at 5% KME probably caused modest thermal stimulation while the 5–6°C rise at 25% KME must have resulted in substantial response to temperature alone. Algal accumulation coefficients at 5 and 25% KME reflected this effect and were, with one exception, more than double control values during all experiments (Table 3). A further complication at the highest KME levels was the succession to a unique diatom flora dominated by *Synedra ulna*, *Diatoma hemalie* and *Fragilaria vaucheriae*. As they may possess slightly higher intrinsic growth rates than those species present in the control trough, a strict nutrient enrichment interpretation of stimulated growth rates at 5 and 25% KME was confounded by the inclusion of taxonomic composition as well as temperature as variables.

Unlike the response of algal growth rates to higher concentrations of KME, the results of exposure to 0.5, 1, and 2% v/v could be attributed primarily to nutrient stimulation. At these high dilutions thermal addition by the effluent was insignificant. However, marked elevation in diatom growth rates at very low KME concentrations were only observed during the summer when river temperature and solar insolation were much higher. If the troughs in the spring experiment had only been selectively shaded from UV-light and not by a 50% neutral density screen, the growth rates measured during that trial would certainly have been higher.

The dramatic nutrient stimulatory response during the summer experient occurred when no measurable increases in the concentrations of the major plant nutrients were observed in the high dilution troughs. This was not unexpected because it has been well established that 1) nutrient limited microbial growth rates are controlled by the level of the intracellular nutrient pool, not by the concentration in the ambient medium (Caperon 1968; Fuhs 1969; Droop 1968) and that 2) the uptake rate of a limiting nutrient increases sharply with small increments of the nutrient when ambient concentrations are very low (Epply et al. 1969). Under conditions of continuously flowing water, nutrient stimulated algal growth in the absence of measurably elevated nutrient levels was not surprising.

An equally plausible explanation for this observation is that the grab sampling program for monitoring nutrients in the troughs missed short-lived but high pulses of nutrients. The rapid uptake ability of algae (McCarthy and Goldman 1979) would certainly have allowed them to take advantage of any nitrogen or phosphorus pulses, with concomitant increases in growth rate. A third possibility was that macronutrient stimulation alone was not responsible for the observed diatom response; some micronutrient, organic growth factor or chelating agent may have been involved. While no data were available to evaluate this possibility we considered it unlikely in view of three lines of evidence strongly suggesting that nitrogen was limiting diatom growth rates.

First, the ratio of available nitrogen to phosphorus in the river water was approximately 1:1 by weight, indicating that of the two, nitrogen was likely to limit growth (Chiaudani and Vighi 1976). Secondly, the concentrations of NH_4^+-N (2–20 $\mu g \cdot L^{-1}$) and $NO_3^- + NO_2^-$-N (5–10 $\mu g \cdot L^{-1}$) were below those known to be required for saturating the growth kinetics of many phytoplankters (Epply and Coatsworth 1968; Epply et al. 1969; MacIsaac and Dugdale 1969). And finally, the limited availability of nitrogen was corroborated by NH_4^+-N uptake rate measurements. Specific uptake rates 2–3 times faster in the control than in the higher effluent treatments demonstrated that the KME was supplying additional nitrogen. Although buffer extractable phosphorus measurements indicated that the KME was also supplying phosphorus, these tests occurred, when SRP in the effluent was an order of magnitude higher than had previously been experienced. We feel that the results of this test would have proven negative if the SRP had been at levels of 20–50 $\mu g \cdot L^{-1}$, that were more characteristic of the effluent.

The initial depression in algal accumulation observed at 25% KME in all the experiments was intriguing. It is not clear whether this phenomenon was due to inhibited growth of the first colonizing diatom species or to passive competition between effluent bacteria and river diatoms for readily colonized sites. While the rapid increase in algal biomass following the initial depression was remarkably similar to the adaptation phenomenon reported by Stockner and Costella (1976) for axenic marine phytoplankton cultures exposed to high concentrations of KME (20–30%), the similarity was only superficial. Taxonomic analysis revealed that the exponential growth phase at high KME concentrations, resulted from a succession to a unique diatom flora. The fact that three of the five predominant species (*Synedra ulna*, *Fragilaria vaucheriae*, and *Nitzschia frustulum*) at 5 and 25% KME have been associated with eutrophic conditions (Lowe 1974) suggests that their success at high KME was due to a competitive advantage at high ambient nutrient level rather than the ability to tolerate some specific inhibitory property of KME.

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Growth and Recruitment in Experimentally Exploited Lake Whitefish (*Coregonus clupeaformis*) Populations

M. C. HEALEY

Department of Fisheries and Oceans, Fisheries Management — Pacific, Resource Services Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

HEALEY, M. C. 1980. Growth and recruitment in experimentally exploited lake whitefish (*Coregonus clupeaformis*) populations. Can. J. Fish. Aquat. Sci. 37: 255–267.

From 1971 to 1978 I conducted an exploitation experiment on lake whitefish (*Coregonus clupeaformis*) in four Northwest Territories lakes. The objective was to test the hypotheses that, when exploited, growth and recruitment would increase in the populations, and that the degree of increase would be proportional to the intensity of exploitation. Significant increases in size-at-age were recorded in the exploited lakes, and the degree and persistence of the increase were proportional to the intensity of exploitation. Good recruitment was stimulated in the heavily exploited lake, and to a lesser degree in the moderately exploited lake. The hypotheses were, therefore, corroborated. Analyses of growth and year-class strength in the unexploited control lake and the lightly exploited lake indicated that natural variation in growth and recruitment, on a similar scale to that stimulated by exploitation, had occurred in the past. The compensatory responses of lake whitefish populations to exploitation appear to be a logical consequence of the natural population regulating mechanisms for the species. The results of my experiment indicate that in unexploited lakes, recruitment and growth of young fish is regulated by the established population of mature fish.

Key words: lake whitefish, growth, recruitment, exploitation, population regulation

HEALEY, M. C. 1980. Growth and recruitment in experimentally exploited lake whitefish (*Coregonus clupeaformis*) populations. Can. J. Fish. Aquat. Sci. 37: 255–267.

De 1971 à 1978, j'ai fait une expérience d'exploitation du grand corégone (*Coregonus clupeaformis*) dans quatre lacs des Territoires du Nord-Ouest. Il s'agissait de vérifier les hypothèses à l'effet que, quand les populations sont exploitées, la croissance et le recrutement augmentent, et que le degré d'augmentation est proportionnel à l'intensité d'exploitation. D'appréciables augmentations de taille pour un âge donné ont été notées dans les lacs exploités, le degré et la persistance de l'augmentation étant proportionnels à l'intensité d'exploitation. Dans le lac intensivement exploité, un bon recrutement est stimulé de façon appréciable, alors qu'il l'est un peu moins dans le lac modérément exploité. Les hypothèses se trouvent donc corroborées. Les analyses de la croissance et de l'abondance des classes d'âge dans le lac témoin non exploité et dans le lac légèrement exploité indiquent qu'il y eut jadis variation de croissance et de recrutement à échelle identique à celle stimulée par exploitation. Les réactions compensatoires des populations de grands corégones à l'exploitation semblent être conséquence logique des mécanismes naturels de contrôle des populations pour l'espèce. Mon expérience indique que dans des lacs inexploités, le recutement et la croissance des jeunes poissons sont réglés par la population déjà établie de poissons matures.

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MODERN fishery management theory is rooted in the concept of a nonlinear relationship between stock size and recruitment (Ricker 1954, 1975; Beverton and Holt 1975; Cushing 1971), such that maximum yield from a fishery occurs at a stock size below that of the unexploited population. In effect, when a fish population is exploited it acts as though trying to compensate for the increased mortality and to regain its former abundance. Although the shape of the curve relating recruitment to adult stock is known to differ among types of fishes (Cushing 1971), the mechanisms by which different fishes compensate for fishing mortality are generally not known. In a review of the dynamics of exploited lake whitefish (*Coregonus clupeaformis*) populations, I concluded that the major response of this species to exploitation was an increase in growth rate which resulted in earlier maturation and shorter generation time. Other parameters related to productivity, such as fecundity and juvenile survival, were too little studied to assess whether or not they also contributed to increased productivity at low stock abundance (Healey 1975).

From 1971 to 1978 I conducted a controlled exploitation experiment on lake whitefish populations in Alexie, Baptiste, Chitty, and Drygeese lakes near Yellowknife,

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Northwest Territories. The objective of the experiment was to measure changes in growth, fecundity, and recruitment resulting from the exploitation of the whitefish. In a previous paper (Healey 1978) I presented evidence that the fecundity of whitefish, and of lake trout (*Salvelinus namaycush*) which were exploited at the same time, increased after exploitation. The purpose of this report is to describe growth and recruitment in the whitefish populations, and to test the hypotheses:

1) When exploited, whitefish show increased rates of growth and recruitment.
2) Increases in growth rate and recruitment are proportional to the intensity of exploitation.

Johnson (1976) and Power (1978) have put forward alternative hypotheses about the structure of whitefish populations and how the number of fish in unexploited populations is regulated. My results provide an insight into the mechanism by which population size is controlled. I shall, therefore, discuss my results in relation to Johnson's (1976) and Power's (1978) hypotheses.

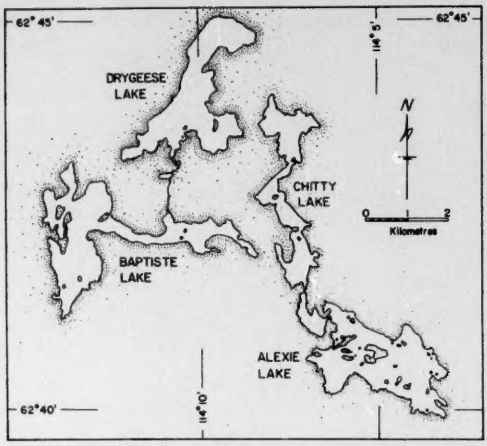

FIG. 1. Alexie, Chitty, Baptiste, and Drygeese lakes near Yellowknife, Northwest Territories.

Materials and Methods

DESCRIPTION OF THE LAKES

Alexie, Baptiste, Chitty, and Drygeese lakes are clustered about 20 km north of the city of Yellowknife in the Northwest Territories (Fig. 1). Their surface areas ranged 304–547 ha, their maximum depths 20–34.5 m, and their mean depths 6.9–14.5 m (Healey and Woodall 1973) (Table 1). Although well within the Precambrian shield, the chemistry of the lakes was more typical of lakes on the margin of the shield (Armstrong and Schindler 1971), except that reactive silicate was low (Healey and Woodall 1973). Phytoplankton, zooplankton, and benthic communities of the lakes were similar, although Alexie tended to be most similar to Chitty and Baptiste to Drygeese (Healey and Kling 1975; Healey 1977). Large fish species in the lakes included lake whitefish, lake trout, northern pike (*Esox lucius*), burbot (*Lota lota*), and lake cisco (*C. artedii*). Small fish species known to occur in the lakes included ninespine stickleback (*Pungitius pungitius*), slimy sculpin (*Cottus cognatus*), lake chub (*Couesius plumbeus*), and troutperch (*Percopsis omyscomaycus*).

SAMPLING AND EXPLOITATION

I surveyed the fish populations in summer and autumn 1971, in spring, summer, and autumn 1972–74, and in autumn 1975, 1976, and 1978 using nylon gill nets of 38, 64, 89, 114, and 140 mm stretched measure. A single unit of effort for survey sampling was a net 91 m long by 1.8 m deep made up in panels 18.2 m long of each of the above five mesh sizes. These nets were set on the bottom in all depths of water greater than 1.5 m. I shall refer to samples taken with these nets as survey samples.

During the summer and autumn of 1973 and 1974 I exploited Alexie, Chitty, and Drygeese lakes using gill nets of 64, 89, and 114 mm stretched measure. These were the most efficient meshes for capturing whitefish in the lakes. A unit of effort for exploitation was a net 137 m long by 1.8 m deep made up in panels 45.7 m long of each of the above three mesh sizes. These nets were set the same way as the survey nets, and I shall refer to samples taken with these nets as harvest samples. I retained Baptiste as an unexploited control lake.

I exploited the three lakes at different rates, Chitty receiving the lowest rate and Drygeese the highest. During 1973 my target was to remove 700 whitefish from Chitty, 2000 from Alexie, and 5000 from Drygeese. During 1974, rather than taking a specific catch, I applied the same fishing effort as in 1973. The sizes of the whitefish populations in the lakes were not known accurately. Sampling in 1971 and 1972, and the decline in catch with fishing in 1973 and 1974 (to be discussed later) indicated that the populations numbered about 7000–15 000 fish with Chitty having the smallest population and Drygeese the largest. Annual rates of exploitation were, therefore, on the order of 10% for Chitty, 20% for Alexie, and 30% for Drygeese.

TABLE 1. Morphometry of Alexie, Baptiste, Chitty, and Drygeese lakes.

Lake	Area (ha)	Depth (m)		Shoreline		Secchi (m)		pH	Conductivity
		Max.	Mean	Length (km)	Development	Range	Mean		μmho/cm
Alexie	420	32	11.7	29.9	4.12	6.0– 9.2	7.6	7.52	98
Baptiste	365	32	11.7	26.1	3.85	5.5– 9.7	7.8	7.33	101
Chitty	305	20	6.9	21.9	3.54	4.8– 8.0	6.3	7.54	102
Drygeese	547	34.5	14.5	21.0	2.53	6.0–10.0	8.3	7.40	109

I recorded fork length to the nearest 0.5 cm, round weight (grams), sex, age, stage of maturity, and stomach fullness of all whitefish captured during survey sampling, and of approximately the first and last hundred whitefish captured during harvesting each year. The remaining whitefish from harvest nets were sorted into 10 cm size categories, and the number and weight of fish in each size category recorded. A subsample of the catch was analyzed for fecundity and stomach contents. I shall present only data on length, weight, and age in this report.

Aging Technique

All whitefish in this study were aged from scales, which is the usual method for aging whitefish. The method is not without its difficulties. Hoagman (1968) showed that two checks, both having characteristics of typical annuli, can form each year on the scales of fish held in captivity. Furthermore, Power (1978) challenged the validity of scale ages for fish older than about 12 yr, showing that many more "rings" can be distinguished on sectioned and burned otoliths than on scales. Since approximately correct aging of the whitefish is important to the results of this study, I shall describe the aging procedure followed, and present some verification of the method.

All scale samples were from midway between the insertion of the dorsal fin and the lateral line, except in the case of small fish which were descaled on removal from the nets. I took scales from wherever possible on descaled fish. The same experienced scale reader read all the scales, and she was provided with no information on the fish other than the scales to be read. I reread a portion of the scales each year, without reference to the age assigned by the principal scale reader, to evaluate agreement between readers. All ages presented in this report are those of the principal scale reader. A few fish could not be aged because the scales collected were regenerate. I assigned ages to these fish based on their length, and the probability distribution of ages at that length.

Many scales were difficult to read. The general criterion for recording an annulus was that it must be continuous in the posterior and lateral portions of the scale. There was considerable opportunity for personal interpretation in applying this criterion. Absolute agreement between myself and the principal scale reader for the subsamples of scales that I reread was only about 60%. Agreement $+/-$ yr, however, was 85% or greater. Most disagreement was among the older fish, and here I tended to age the fish older than the principal scale reader. Scales read twice by both the principal scale reader and myself generally resulted in less than 15% change in the original ages assigned. This comparison indicates that the ages assigned to fish may be in error by a minimum of $+/-$ 1 yr. Two sources of information suggest that the degree of error is probably not much greater than this. Firstly, I tagged and released 28 whitefish in Alexie and Chitty lakes in 1972. From their scales, these fish ranged in age from 7–12 yr old at the time of tagging. I recaptured eight of the tagged fish 1–4 yr later. The principal scale reader aged all the fish older when recaptured than when first captured. Four fish were aged the correct number of years older. Two were aged too old by 1 yr, and two were aged too young by 1 yr, relative to the years between tagging and recapture. Fish in the 7–12 yr age-classes, therefore, were still laying down about one discernible annulus per year. Few fish aged older than 12 yr were captured in the lakes. Secondly, strong year-classes were recognizable when recruited in Baptiste and Drygeese. These year-classes could be followed in successive sampling years, progressing in age by about 1 yr each year.

Some whitefish were, therefore, probably assigned to an age-class one year older or younger than their true age-class. It seems unlikely, however, that the fish were grossly underaged as Power (1978) has suggested for some populations. Aging errors were symmetrical in the small sample of tagged fish. This does not necessarily mean, however, that aging errors were equally distributed above and below the true age in all samples. Aging errors based in one direction could affect the interpretation of my results. The effects of exploitation were most noticeable among young fish, however, where aging is most reliable.

Analytical Techniques

I examined growth by comparing measured length- and weight-at-age before and after exploitation. In addition, I examined historical variation in growth by back calculating length-at-annulus-formation from scales. In back calculating lengths, I used only scales on which the principal scale reader and I agreed as to age. Measurements were made from the focus to each annulus up to the sixth and to the scale margin along the posterior axis of the scale. I estimated fish length at annulus formation from a least squares regression of fish length on scale radius.

I examined recruitment by comparing age frequency histograms for the 7 yr of sampling, and by calculating relative year-class contribution to the populations. In unexploited Baptiste and lightly exploited Chitty lakes, I estimated the relative year-class contribution for 15 year-classes by assuming little or no effect of fishing on the population. Consider the following relationships:

(1) $C_{y,a} = k_a P_{y,a} E$

(2) $P_{y,a+1} = P_{y,a} \exp(-m_a - f_a) \cong P_{y,a} \exp(-m_a)$

(3) $f_a \cong 0$

$C_{y,a}$ is the catch of year-class y at age a.
k_a is the catchability of fish aged a, assumed the same for all year-classes.
$P_{y,a}$ is the population of fish of year-class y that are age a.
E is the fishing effort.
m_a is the instantaneous annual rate of natural mortality of fish aged a, assumed constant for all year-classes.
f_a is the instantaneous annual rate of fishing mortality on age a, assumed very small.
thus from (1) and (2) above

$$(4)\quad \sum_{a=1}^{n} \frac{C_{y,a}}{E} \cong P_{y,1}(k_1 + k_2 e^{-m_1} + k_3 e^{-(m_1+m_2)} + \ldots + k_n e^{-(m_1+m_2+\ldots+m_{n-1})})$$

That is to say, provided mortality due to fishing is negligible, the sum of catch-per-unit-effort (C/E) for a year-class over all the years it is present in the population is proportional to the size of the year-class at recruitment. The catch at any age is also proportional. By summing over all ages, however, random variations in catchability from year to year are less likely to influence the interpretation of year-class strength.

The study was not long enough to follow even one year-class completely through the population. To expand partial

TABLE 2. Catch (C) and catch per unit effort (C/E) during survey and harvest fishing in the four lakes each year.

	Baptiste		Chitty				Alexie				Drygeese			
	Survey		Survey		Harvest		Survey		Harvest		Survey		Harvest	
Year	C	C/E	C	C/E	C	C/E	C	C/E	C	C/E	C	C/E	C	C/E
1971	48	9.6	51	10.2	—	—	52	10.4	—	—	96	19.2	—	—
1972	106	15.1	88	11.0	—	—	92	11.5	—	—	107	15.3	—	—
1973	71	10.1	91	15.2	645	17.0	64	10.7	1979	14.3	69	9.9	4700	8.9
1974	121	13.4	81	9.0	839	22.1	53	5.9	1203	8.7	51	5.7	1605	3.0
1975	114	11.4	59	5.2	—	—	113	7.7	—	—	159	8.2	—	—
1976	219	10.0	52	4.9	—	—	57	5.3	—	—	128	10.2	—	—
1978	85	15.8	51	10.0	—	—	48	9.3	—	—	50	9.8	—	—

year-class contributions I calculated the average contribution of each age-class to the catch for all samples prior to exploitation. From the average year-class contribution at each age, I extrapolated the catch of a particular year-class over part of its life span to an estimate of the probable contribution over its whole life span.

In more heavily exploited Alexie and Drygeese lakes I estimated the strength of a few year-classes from the decline in catch of each age class with fishing. The regression of C/E on cumulative catch for each age-class permits an estimate of the total numbers in that age-class, provided fishing is sufficiently intense to significantly reduce the abundance of the age-class. Ricker (1975) summarized the method, called the removal method, and its underlying assumptions. The number of fish in each age-class is a rough measure of year-class strength with the proviso that each succeeding age-class will number less than the preceding one due to natural mortality. The number of fish in all age-classes for which estimates could be made was also a measure of the total fishable stock. From these estimates I was able to infer total population size in Alexie and Drygeese.

To employ the removal method I needed the age structure of the catch throughout each harvest period. Age structure of the harvest catch was recorded for only the first and last 100 fish captured. Other fish captured were recorded by size category. I estimated the age composition of the unaged catches from the probability distribution of ages within each size category.

Both the back calculated lengths, and the estimates of year-class strength provided an historical perspective not available from the raw catch data. This historical perspective was important to the interpretation of changes in growth and recruitment that followed harvesting in Chitty, Alexie, and Drygeese lakes.

Results

EFFECTS OF EXPLOITATION ON CATCH

In sampling Baptiste I attempted to gather the necessary information on abundance, age structure, and size with as little disturbance to the fish community as possible. After 1971, I regulated effort so as to capture approximately 100 whitefish each year. This was reasonably successful except in 1976 when I captured more than 200 whitefish (Table 2). C/E of whitefish in Baptiste ranged 9.6–15.8 between 1971 and 1978, and averaged 12.2 ± 2.256 (SD). C/E was similar in Chitty and Alexie during the years before exploitation, averaging 10.6 and 11.0, respectively. Drygeese appeared to have a more dense population, C/E averaging 17.2 during 1971 and 1972 (Table 2).

Exploitation had little immediate effect on the abundance of whitefish in Chitty Lake (Table 2). During 1973, the first year of exploitation, C/E in survey nets was about 40% higher than in 1971 and 1972. Harvest net C/E was also high. During 1974, survey net C/E was slightly below 1971–72 levels, but harvest net C/E was higher than in 1973. For 2 yr following exploitation, however, survey net C/E was less than half that in 1971–72. In 1978, survey net C/E was again similar to that in 1971–72.

Exploitation did have a direct effect on whitefish abundance in Alexie (Table 2). Survey net C/E in 1973 was comparable with 1971 and 1972, and harvest net C/E was 14.3, slightly less than in Chitty. During 1974, however, survey net C/E was about half that in 1971–72 and harvest net C/E was about 60% of that in 1973. Survey net C/E remained low during 1975 and 1976 but in 1978 had increased nearly to the 1971–72 level. Alexie and Chitty showed similar patterns of changing C/E throughout the study, therefore, with the exception that C/E dropped during the second year of exploitation in Alexie but not in Chitty.

Exploitation had the greatest effect on catches in Drygeese (Table 2). During 1973, survey net C/E dropped to 58% of that in 1971–72, and in 1974 to only 33% of the 1971–72 level. Harvest net C/E in 1973 was only 8.9, considerably less than harvest net C/E in Chitty and Alexie, both of which had less dense initial populations. In 1974 harvest net C/E was further reduced to 3.0. Despite the large reduction in population size in Drygeese during exploitation, catches began to recover in the first year following exploitation. Survey net C/E increased to 8.2 in 1975, and to 10.2 in 1976. There was no further increase in C/E in 1978, although C/E did increase in the other lakes (Table 2).

Further indication of the impact of exploitation on the whitefish populations of Chitty, Alexie, and Drygeese lakes is shown by the age structure of the catch at the beginning and end of 2 yr of exploitation (Fig. 2).

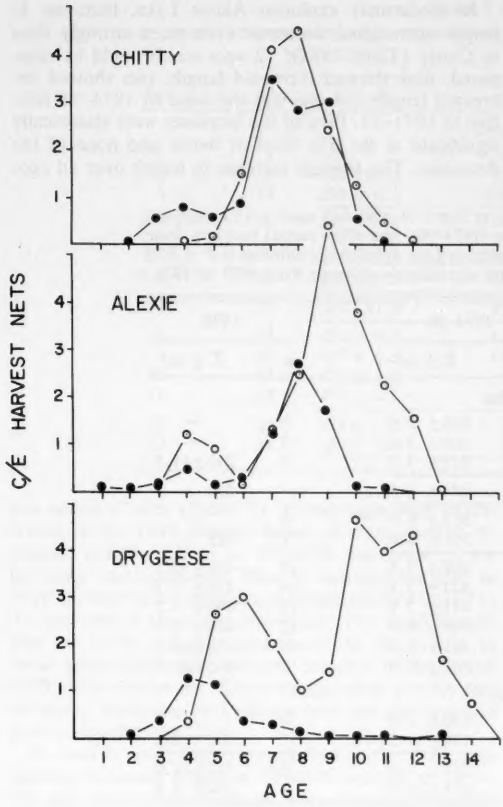

FIG. 2. Age composition of the harvest catch in Chitty, Alexie, and Drygeese lakes at the start of harvesting in 1973 (○) and at the end of harvesting in 1974 (●).

In Chitty, only relatively small differences were evident. Age 3 and 4 fish were absent from the catch at the start of exploitation, but were present in small numbers at the end. Older fish were slightly less abundant in the catch at the end of exploitation than at the start. These changes could result from year-to-year fluctuations in catchability, and cannot be interpreted with certainty as results of the exploitation. The changes in Chitty are, however, consistent with the expected effects of fishing on age structure, and with what occurred in the other lakes. In Alexie, changes in age structure were more dramatic. Fish aged 10–12, abundant at the start of exploitation, were virtually gone at the end, and fish aged 9 were considerably reduced in abundance. As in Chitty, young age-classes, not present in the catch at the start of exploitation, were present at the end. In Drygeese, exploitation severely reduced all age-classes above 5, while ages 3 and 4 increased in C/E (Fig. 2).

I interpret the reduction in C/E of older age-classes in Alexie and Drygeese to be a direct result of exploitation. The smaller reductions in Chitty may also have been due to exploitation, but this is not certain. The increased catch of young age-classes may also be a result of exploitation, and this is discussed later. Here I note only that exploitation produced measurable changes in the whitefish populations of two of the lakes, and that the degree of change was proportional to the intensity of exploitation.

EFFECTS OF EXPLOITATION ON GROWTH

To test the null hypothesis of no increase in growth rate following exploitation I compared the mean lengths of fish captured before and after exploitation for each age-class. In making this comparison I considered fish captured in 1971–73 to be representative of conditions before exploitation. Exploitation in 1973 was mainly in summer and autumn, and it seemed unlikely that any response would be measurable in the fish sampled that year. Fish captured in 1974–76 I took to be representative of conditions following exploitation. Fish sampled in 1978 could represent either recovery or continued response to exploitation, so I did not combine 1978 lengths with those from 1974 to 1976 (Table 3). My principal reasons for combining 1971–73 data and 1974–76 data were to increase sample size in the youngest and oldest ages, and to smooth out any random year-class variations in growth rate not related to exploitation.

In unexploited Baptiste, increases and decreases in length-at-age over the study period were about equal (Table 3). Of 12 ages which could be compared, five showed increased length, six showed decreased length, and one was the same in 1974–76 relative to 1971–73. One of the increases in length and two of the decreases were statistically significant at the 5% level (Student's t-test). Nine ages were represented in the 1978 sample from Baptiste. Of these, five showed greater average length and four lesser average length relative to 1974–76. None of these differences was statistically significant. All but one of the ages sampled in 1978 showed greater average length relative to 1971–73, but none of the differences was significant. Although there was a slight trend toward increasing size at age in Baptiste, therefore, this could be due to sampling error.

In lightly exploited Chitty Lake, increases in length outweighed decreases over the study period (Table 3). Of 12 ages which could be compared, seven showed increased length, three showed decreased length, and two were the same in 1974–76 relative to 1971–73. Five of the increases were statistically significant at the 5% level or better (one tailed t-test), and none of the decreases (two tailed t-test) (Table 3). The average increase over all ages was about 5 mm. Nine ages were represented in the 1978 sample from Chitty. Of these, two showed greater average length and seven lesser average length relative to 1974–76. Three of the de-

creases in average length and one increase were significant at the 5% level (two tailed *t*-test). Comparing 1978 with 1971–73, four ages showed increases in length and five showed decreases. One of the decreases was significant at the 5% level (two tailed *t*-test) (Table 3). The results for Chitty suggest that growth was better for a short period following exploitation, but returned rather quickly to pre-harvest levels.

In moderately exploited Alexie Lake, increases in length outweighed decreases even more strongly than in Chitty (Table 3). Of 12 ages which could be compared, nine showed increased length, two showed decreased length and one was the same in 1974–76 relative to 1971–73. Five of the increases were statistically significant at the 5% level or better and none of the decreases. The average increase in length over all ages

TABLE 3. Sample size and mean fork length ± SE (mm) of whitefish aged 1–12 y captured before exploitation (1971–73), after exploitation (1974–76), and after partial recovery from exploitation (1978). Adjacent values which are underlined are significantly different at $P < 0.05$ (Student's *t*-test). (*Indicates that 1978 values are significantly different from 1971 to 1973.)

	1971–73		1974–76		1978	
Age	*n*	$\bar{L}$ ± SE	*n*	$\bar{L}$ ± SE	*n*	$\bar{L}$ ± SE
			Baptiste			
1	1	165	4	168± 5.9	0	—
2	5	207± 7.4	14	201± 3.4	0	—
3	6	209± 4.2	8	252± 3.7	5	232±11.5
4	4	304± 6.9	11	284± 5.1	1	230
5	9	319±15.9	24	305± 6.8	0	—
6	12	399±10.2	26	351± 8.0	1	425
7	42	408± 4.5	49	408± 4.8	10	428± 7.3
8	67	429± 3.4	65	424± 2.8	34	434± 3.1
9	53	434± 2.6	60	437± 2.9	12	448± 3.9
10	19	437± 4.7	44	445± 4.0	12	444± 5.3
11	3	442±10.9	19	441± 4.4	6	450±10.1
12	1	430	5	482±30.5	2	462±22.5
			Chitty			
1	9	170± 3.2	7	170± 3.0	0	—
2	11	202± 5.8	8	196± 3.2	13	208± 2.5
3	9	248± 7.9	27	258± 3.5	8	252±11.0
4	14	272± 8.6	23	295± 5.8	7	264± 9.9
5	14	322±11.3	42	312± 7.7	0	—
6	41	368± 3.7	13	372±11.3	3	338± 7.3*
7	84	395± 3.1	38	407± 3.4	2	368±12.5
8	103	413± 2.1	73	419± 2.6	5	399± 2.9
9	85	421± 2.3	59	430± 3.0	4	428± 9.7
10	46	426± 3.0	27	440± 4.7	6	433±12.0
11	13	420± 6.3	7	420±11.6	0	—
12	2	440± 0.0	3	432± 9.4	2	460± 5.0
			Alexie			
1	1	180	7	174± 8.2	0	—
2	1	180	3	196± 5.8	1	200
3	5	239± 4.9	14	264± 4.6	5	257± 8.9
4	23	271± 6.4	16	298± 6.8	3	283±14.8
5	15	307± 9.0	13	325± 7.9	8	336±15.3
6	9	327±12.3	23	371± 7.1	5	404±14.7*
7	44	417± 4.1	53	405± 4.6	4	408±18.9
8	74	426± 2.6	85	426± 2.4	7	442± 4.5
9	103	433± 2.3	106	442± 2.1	2	432± 2.5
10	72	436± 2.3	43	448± 3.3	5	450± 5.7
11	36	446± 4.3	35	452± 2.9	1	465
12	19	453± 6.3	6	461± 8.2	4	458±15.3

TABLE 3 (*Concluded*)

Age	1971–73		1974–76		1978	
	n	$\bar{L} \pm$ SE	*n*	$\bar{L} \pm$ SE	*n*	$\bar{L} \pm$ SE
			Drygeese			
1	1	170	3	171± 7.2	1	175
2	2	175±10.0	72	185± 1.7	0	—
3	17	210± 6.1	107	232± 3.1	0	—
4	26	261± 6.1	113	279± 2.8	11	291± 4.7*
5	46	292± 4.7	115	306± 3.0	11	315± 8.5*
6	42	322± 5.9	67	338± 4.1	16	393± 7.8*
7	21	364±11.8	22	389± 9.8	8	426± 5.6*
8	32	432± 9.3	13	432±13.9	0	—
9	45	475± 4.9	19	483± 4.3	0	—
10	63	473± 2.0	9	478± 4.1	0	—
11	39	474± 4.1	9	493± 5.0	0	—
12	31	478± 3.1	3	458±16.7	0	—
13	14	482± 5.8	3	520±16.0	0	—

was about 12 mm (Table 3). Eleven ages were represented in the 1978 sample. Seven of these showed increased length relative to 1974–76, but none of the increases was significant. Nine of the ages sampled in 1978 showed increased average length relative to 1971–73, and one of these was significant. The small sample size for 1978 probably contributed to the failure to show more significant increases between 1971–73 and 1978. The results for Alexie suggest that growth rate increased following exploitation and this increase was maintained through 1978.

In heavily exploited Drygeese Lake, 11 of 13 ages showed increased length in 1974–76 relative to 1971–73, and seven of the increases were statistically significant. The average increase in length over all ages was 12 mm, the same as in Alexie (Table 3). Five ages were represented in the 1978 sample, and all showed increased length over 1974–76. Lengths at two ages were significantly greater in 1978 than in 1974–76, and lengths at four ages significantly greater than in 1971–73. The Drygeese results suggest that growth rate increased following exploitation and was still increasing 4 yr after exploitation stopped (Table 3). Comparing Chitty, Alexie, and Drygeese, following exploitation there was a small, short-lived increase in length-at-age in Chitty, a larger and more persistent increase in Alexie and a large and continuing increase in Drygeese.

Average weight at age showed a similar pattern of changes in relation to exploitation as did length (Table 4). Round weight, because of water loss, stomach contents, maturity, and other reasons, was more variable than length, so that fewer changes were statistically significant. There is nothing in the data on weight, however, to contradict the conclusions from the data on length.

These data corroborate the hypotheses that exploitation of whitefish will stimulate an increase in growth, and that the degree of increase will be proportional to the intensity of exploitation. The absolute increases in size were not large, however, even in Drygeese Lake where the fishable population was reduced by more than 70% and total population by an estimated 50%. The increase in size of whitefish in Drygeese after exploitation was about half the increase that I expected from comparing the pre-harvest size-at-age, with the potential maximum size-at-age for whitefish (Healey 1975).

Although the observed increases in length and weight appeared to be consequences of exploitation, it is possible that they represent fortuitous natural variation. To interpret such changes properly requires an appreciation of natural variation in size-at-age in the population prior to exploitation. Back calculated lengths-at-annulus-formation from scales revealed considerable variation in growth among year-classes prior to exploitation (Fig. 3). In general, all year-classes within a lake were the same length at age 1, but 1-yr-old fish differed in length between lakes. For all other ages there was variation in size among year-classes within a lake. The degree of variation increased with age until by age 6 (the oldest age to which I carried back calculation) the difference between the slowest growing and fastest growing year-classes ranged from 78 mm in Chitty to 100 mm in Baptiste (Fig. 3).

The pattern of increase and decrease in growth rate among year-classes was similar from lake to lake. The same three year-classes, 1964–66 showed the fastest growth rate in Baptiste, Chitty, and Alexie lakes, while the three fastest growing year-classes were 1962–64 in Drygeese. Year-classes 1967–70 grew slowly in all the lakes. Year-classes 1971 and 1972 showed increased growth rate in all but Chitty Lake suggesting the start of another series of rapidly growing year-classes (Fig. 3). That good and poor growth were features of year-classes rather than years indicates that some population

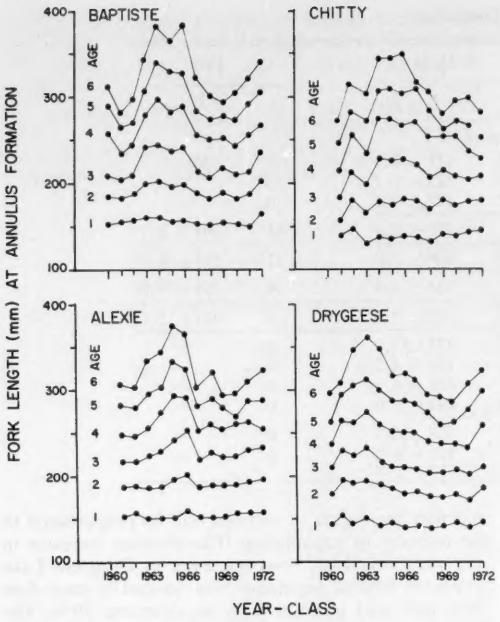

FIG. 3. Average fork length at ages 1–6 for year-classes 1960–72 in Baptiste, Chitty, Alexie, and Drygeese lakes. Lengths were back calculated from scale measurements.

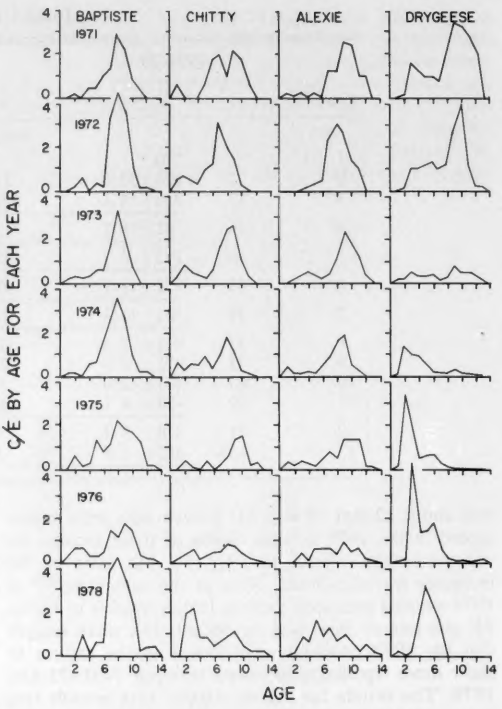

FIG. 4. Age structure of the catch in Baptiste, Chitty, Alexie, and Drygeese lakes for 1971–76 and 1978.

related phenomenon was influencing growth rather than simple year-to-year variation in environmental conditions or food supply.

EFFECTS OF EXPLOITATION ON RECRUITMENT

From the C/E of each age in each mesh I calculated the age structure of the fishable population each year for the range of meshes in the survey nets. These age structures are not the true age structure of the population because the ages differ in catchability. In general, the younger age-classes are greatly under represented. Assuming catchability did not change appreciably from year to year, however, the age structures should be comparable, and changes in sample age structure from year to year should reflect recruitment and survival in the populations. Changes in size-at-age discussed above will influence catchability and this will have to be considered in interpreting the sample age structures, particularly for Drygeese.

In Baptiste, ages 7–9 had the greatest C/E in most years, while fish younger than 6 yr and older than 10 yr had generally low C/E. From 1971 to 1976 the C/E of fish older than 10 and younger than 6 tended to increase, suggesting aging of the mature fraction of the stock and recruitment of young fish. In particular, there appeared to be a good recruitment of young white-fish in 1975 and 1976, and this was accompanied by an obvious decline in the C/E of fish in the 7–9 age-group (Fig. 4). The young fish recruited in 1975 and 1976 were still apparent in 1978. Their contribution to the catch was low, however, in spite of their larger size and presumably greater vulnerability to the range of meshes fished, and in spite of the high average C/E in 1978. Catch of fish aged 7–9 in 1978 was higher than in any year except 1972 (Fig. 4).

From 1971 to 1976 the population in Baptiste appeared to be following an orderly process of aging and decline in abundance of the older, mature fraction of the stock, accompanied by recruitment of young fish, particularly in 1975 and 1976. In 1978, however, this pattern was broken, and the age structure of the catch was similar to that in 1972.

In Chitty Lake, as previously noted, exploitation in 1973 and 1974 did not have a dramatic effect on age structure or C/E. Gillnet catch in Chitty was at first dominated by fish aged 7–9. Like Baptiste, Chitty underwent an orderly process of aging and decline in abundance of mature fish accompanied by recruitment of young fish. Unlike Baptiste, the pattern did not break

TABLE 4. Mean weight (g) of whitefish aged 1–12 captured before exploitation (1971–73), after exploitation (1974–76), and after partial recovery from exploitation (1978).

	Baptiste			Chitty			Alexie			Drygeese		
Age	71–73	74–76	1978	71–73	74–76	1978	71–73	74–76	1978	71–73	74–76	1978
1	50	44	—	49	46	—	55	49	—	50	50	100
2	83	76	—	80	79	118	55	78	100	45	58	—
3	87	167	148	167	189	218	152	202	208	94	134	—
4	300	247	140	249	304	231	217	308	300	215	240	296
5	419	325	—	435	370	—	344	425	497	269	324	370
6	798	511	990	654	669	460	497	633	914	378	448	780
7	885	824	1054	786	902	635	930	867	918	591	751	975
8	1036	936	1012	922	982	816	976	1027	1210	1031	1112	—
9	1079	992	1141	945	1076	1040	1030	1157	1185	1330	1491	—
10	1127	1076	1107	970	1146	1065	1084	1229	1324	1306	1431	—
11	1133	1045	1090	960	1001	—	1139	1301	1370	1359	1664	—
12	930	1480	1305	1075	1225	1340	1236	1396	1298	1322	1325	—

down in Chitty, and 1978 samples indicated a large recruitment of young fish (Fig. 4).

In Alexie, exploitation significantly reduced the abundance of older age-classes (Fig. 2). After exploitation the surviving mature fish showed the same kind of aging and decline in abundance described for Baptiste and Chitty. Recruitment of young fish occurred in Alexie also, but it was a moderate recruitment of several year-classes rather than a large recruitment of a few year-classes as in Chitty (Fig. 4).

In Drygeese, exploitation virtually eliminated the mature fish. This was followed by a very large recruitment of young whitefish age 0+ and 1+ at the time of exploitation. This heavy recruitment was evident through 1978 as a strong peak in abundance (Fig. 4).

The increase in size-at-age following exploitation in Chitty, Alexie, and Drygeese is not a sufficient explanation for the increased catch of young fish. Size increases occurred at all ages, yet increased C/E was specific to the youngest ages, with little or no increase in C/E of intermediate ages. Nor were increases in C/E proportional to increases in size-at-age as would be expected if the increased catch were a result of increased size at age.

Further information on recruitment to the populations in Baptiste and Chitty is provided by the estimates of relative year-class strength from equation (4). Relative year-class strength for year-classes 1960–74, varied 3–5 fold in these lakes (Fig. 5). Furthermore, periods of good and poor year-class strength tended to coincide in the two lakes. Although their strength could only be approximated because of the few years during

TABLE 5. Rates of decline in C/E with increase in cumulative catch for each age during harvesting in Alexie and Drygeese, and population estimate for 1973 based on 1973 catch and 1974 catch. Rate of C/E decline is the slope of C/E regressed on cumulative catch over the period of harvest fishing each year. The method for making each population estimate is discussed in the text.

Alexie						Drygeese					
	Rate of C/E decline		1973 Population estimate				Rate of C/E decline		1973 Population estimate		
Age	1973	1974	From 1973 fishing	From 1974 fishing	Mean	Age	1973	1974	From 1973 fishing	From 1974 fishing	Mean
3	+0.0168	−0.0069	(161)	161	161	3	+0.0015	−0.00004	—	—	—
4	+0.0017	−0.0096	(237)	237	237	4	−0.0004	+0.0002	1036	1584	1310
5	−0.0009	−0.0082	860	184	522	5	−0.0036	−0.0013	792	862	827
6	−0.0181	−0.0062	10	238	124	6	−0.0051	−0.0026	586	625	606
7	−0.0044	−0.0078	287	765	526	7	−0.0065	−0.0068	431	484	458
8	−0.0027	−0.0068	934	713	824	8	−0.0025	−0.0097	435	402	418
9	−0.0047	−0.0074	1170	587	878	9	−0.0026	−0.0128	659	528	594
10	−0.0035	−0.0150	1147	484	816	10	−0.0040	−0.0117	864	798	831
11	−0.0071	−0.0120	330	196	263	11	−0.0060	−0.0139	634	626	630
12+	−0.0153	—	125	70	98	12	−0.0072	—	424	418	421
						13	−0.0044	—	217	198	207
						14+	−0.0059	—	62	59	60

which they occurred in the catches, the 1975 year-class in both lakes, and the 1976 year-class in Chitty, appeared to be strong year-classes. This indicates that a series of 3–4 exceptional year-classes may occur every 10–12 yr, with the intervening 6–8 yr characterized by poor to moderate year-class strength.

In Alexie and Drygeese, age-classes 4 and older were sufficiently reduced by fishing in 1973 and 1974 to permit a population estimate by the removal method (Table 5). The estimate for 1973 refers to the population prior to fishing, but the estimate for 1974 refers to the population remaining at the end of fishing in 1973. I, therefore, added the catch in 1973 to the estimate in 1974 to obtain a second estimate of the population of each age-class prior to fishing (Table 5). This second estimate is biased low by the natural mortality occurring in the populations between harvest periods. Nevertheless, the relative estimates of population size based on the 1974 fishing should be unbiased, and indicate the degree of confidence that may be placed in the estimates of abundance.

Differences between the 1973 and 1974 estimates of preexploitation population were much greater in Alexie than in Drygeese. In Alexie, the two estimates totaled 5100 and 3474, respectively, and the correlation between the estimates for individual ages was only +0.449 (Table 5). In Drygeese, on the other hand, the two estimates totaled 6140 and 6584, respectively, and the correlation between estimates for individual ages was +0.925 (Table 5).

Even though the population estimates for individual ages in Alexie were inconsistent, they do suggest a pattern of year-class strength similar to that for Baptiste and Chitty, with good year-classes in the mid-1960s and poor year-classes in the late 1960s. In Drygeese the variation in year-class strength appeared to be small and out of phase with that in the other lakes. Good year-classes occurred in Drygeese in 1962 and 1963, and possibly also in 1968 and 1969. The poorest year-class in Drygeese appeared to be 1965, one of the best in the other lakes.

Discussion

Within the 8-yr time frame of my experiment, the results appear to corroborate the two hypotheses presented. Average size at age increased after exploitation in all the exploited lakes, and the degree and persistence of the increase were proportional to the intensity of exploitation. In the most heavily exploited lake, exploitation was immediately followed by good recruitment of two year-classes, and recruitment appeared to be stimulated in moderately exploited Alexie as well. These results, particularly those for Drygeese Lake, are qualitatively similar to Miller's (1947, 1956) results for a similar experiment on Pigeon Lake, Alberta. In Miller's experiment, an already high rate of exploitation on Pigeon Lake was allowed to increase still further. The resulting reduction in standing stock was accompanied by a significant increase in growth rate, and a large recruitment of young whitefish. The similarity between Miller's results and mine support the interpretation that increases in growth and recruitment are a consequence of exploitation.

The straightforward interpretation of my results, however, is partially confounded by natural fluctuations in growth and year-class strength revealed by the back calculation of length and the analysis of relative year-class strength. Other authors have amply demonstrated the existence of similar fluctuations in year-class strength in whitefish populations (Miller 1952; Kennedy 1954; Lawler 1965), and have related year-class strength to such phenomena as seasonal temperature regimes, and high winds at spawning time. Furthermore, Miller (1952) demonstrated correspondence among good and poor year-classes in a variety of prairie lakes, and implicated weather as a synchronizing mechanism. If weather conditions are, in part, responsible for good and poor year-classes, it is not surprising that year-class strengths were positively correlated in Baptiste, Chitty, and Alexie. Year-class strengths in Drygeese, however, appeared not to be correlated with the other lakes.

Variation in growth rate among year-classes has received much less attention than variation in year-class strength. Kennedy (1943) back calculated length-at-annulus-formation for Lake Opeongo whitefish, and demonstrated differences in growth among year-classes. Edsall (1960), however, found similar rates of growth among several year-classes of whitefish from Munising Bay, Lake Superior. Kennedy (1943) suggested that the decline in growth of Lake Opeongo whitefish between year-classes 1926–31 was due to recovery of the stock from commercial fishing during the first world war. This seems unlikely, as the fishing in Lake Opeongo was light and irregular, and occurred a decade prior to the appearance of the 1926 year-class. Variation in growth rate among year-classes in Lake Opeongo and in my lakes prior to exploitation probably reflects some natural population phenomenon.

Since variation in growth and recruitment apparently occurs naturally in unexploited lakes, could the changes in growth and recruitment observed during my experiment simply result from natural fluctuations rather than the effects of exploitation? This question is particularly important because the historical pattern of variation in these parameters suggests alternating periods of good and poor growth and recruitment, and because variations in growth rate prior to exploitation were as great as those occurring after the exploitation.

Natural fluctuations in growth rate are an insufficient explanation for the larger size of fish following exploitation. In most instances where a significant increase in length-at-age was recorded after exploitation, the year-classes from which average length before exploitation was calculated were among the faster growing year-classes, while those from which average length after exploitation was calculated were among the slower

growing. Rather than accounting for the increase in size at age after exploitation, therefore, historical variation in growth rates may have made it more difficult to demonstrate the increase.

Published data on growth in whitefish suggest a common maximum growth rate for whitefish stocks, a rate achieved by all heavily exploited stocks for which there is information (Healey 1975). I expected growth in Drygeese Lake to approach this maximum. By 1978, however, whitefish aged 4–7 from Drygeese Lake averaged 8–16 cm shorter than fish of the same ages growing at the maximum rate. The question remains whether continued exploitation in Drygeese, or the other lakes, would eventually have resulted in this maximum growth rate. It is possible that, in Drygeese Lake, the large number of young fish recruited immediately after exploitation partially inhibited the growth response.

The possible contribution of natural fluctuations in year-class strength to the recruitment of young fish after exploitation is more difficult to evaluate. If periods of good and poor recruitment are somewhat cyclic, then the time was appropriate for a period of good recruitment in the lakes. In Drygeese, however, the C/E of the 1972 and 1973 year-classes between ages 2–6 was very large. Unless the catchability of young fish increased dramatically after exploitation, this high C/E must indicate a large recruitment. Catchability of young fish did increase slightly during exploitation in Drygeese and Alexie lakes, but this increase was insufficient to explain the very high C/E values in Drygeese. Year-classes 1975 and 1976 in Chitty Lake, and 1972–75 in Alexie Lake appear strong, and this may have been partly a consequence of natural events. Exploitation may also have contributed to the recruitment of these year-classes, however, by speeding the decline in abundance of older fish.

Predators were captured in moderate numbers during exploitation of the lakes. Power (1978) has suggested that predation and cannibalism are important population regulating mechanisms among long lived northern fish populations and it is conceivable that reduced predation may have affected recruitment in the lakes. Lake trout was the only predator clearly reduced in abundance by the exploitation, while C/E of pike and burbot remained the same or increased during the years of exploitation. Lake trout occasionally preyed on lake whitefish, but whitefish made up less than 10% of their diet by volume. The principal prey of lake trout and of the other predators were cisco and stickleback. Cisco and stickleback are both planktivores. They could prey upon whitefish larvae at hatching, and later might compete with young whitefish during their first summer when whitefish also feed on plankton (Hoagman 1973). The possible consequences of a reduced lake trout population for whitefish stocks are, therefore, both positive and negative. The positive effect of reduced trout predation after exploitation could easily be offset by the negative effect of increased predation and competition from cisco and stickleback. Finally there is the qualitative similarity between my results, and those from Pigeon Lake (Miller 1956), which has no trout. The similarity in results would be hard to explain if my results were a consequence of lake trout removal.

Natural variations in growth and recruitment and predator removal cannot, therefore, account for the changes in growth and recruitment observed during this study.

Evidence presented here, and elsewhere (Healey 1975), leaves little doubt that when whitefish are exploited their growth rate increases. Also, good recruitment can apparently be stimulated by exploitation, and the average fecundity of the fish may increase (Healey 1978). Whitefish thus demonstrate a variety of mechanisms by which the added mortality caused by fishing may be mitigated. Of these mechanisms, increased growth rate appears to be the most important overall. The expression of these mechanisms in my study also provides insight into the regulation of whitefish population size in unexploited lakes.

Three observations from this experiment appear particularly relevant to the natural regulation of population size in whitefish. Firstly, variations in growth prior to exploitation were a feature of year-classes rather than years. Secondly, periods of good recruitment were associated with declines in adult stock, and the recruited year-classes were already present in the population at the time when adult stock declined. Thirdly, the catchability of young whitefish apparently increased after the removal of the adult stock in Drygeese and Alexie lakes.

It is a feature of gill net catches of lake whitefish that small young fish are relatively rare, while fish of intermediate size and age are abundant. Obviously this cannot represent the true age structure of the population, at least not an age structure that would persist for any length of time. Three hypotheses have been proposed to account for this typical gill net catch curve. Firstly, young fish are as abundant in the general population as older fish, but are underrepresented in the catch because of gill net selectivity and lower efficiency of small meshes (Ricker 1975). Secondly, the age structure results from gross errors in aging (Power 1978). Thirdly, young fish are forced into marginal habitats, or are otherwise controlled in their distribution and movements by the older fish (Johnson 1976). Gill net efficiency, and aging problems certainly contribute to, and accentuate, the artificial shape of the gill net catch curve. The three observations noted above, however, are more consistent with some of Johnson's (1976) ideas, although my interpretation of his results does not correspond exactly with his.

The increase in catchability of young fish, and the rapid recruitment to the fishable stock of year-classes already in the population when the adult stock is reduced are consistent with the hypothesis that an abundant population of old fish somehow suppresses the younger fish. Such suppression might take the form of relegation to marginal areas or habitats, as suggested

by Johnson (1976), or might involve inhibition of normal exploratory and foraging activity. Either mechanism would significantly reduce the catchability of young fish by standard gill net gangs, and such inhibition would probably also result in high juvenile mortality. Removal or death of the adult fish would permit the surviving young fish to invade the areas formerly occupied by the older fish, or to become more active and hence become more vulnerable to gill nets. Recently produced year-classes might also survive well, and produce several strong year-classes.

If good recruitment in whitefish populations depends on the death of adult fish then pulses of recruitment would occur at intervals approaching the life span of the fish. Observing such a recruitment phenomenon in an unexploited population of long lived fish would be a rare event. Johnson (1976), for example, presented many age frequency distributions for whitefish, only a few of which show evidence of recruitment. In Drygeese Lake, however, rapid removal of the old fish apparently stimulated the recruitment of two strong year-classes which also had high catchability. In Chitty and Alexie good recruitment was associated with a decline in the adult population, but this occurred some time after exploitation. In Baptiste, older fish appeared to be getting scarcer in 1975 and 1976, and there was indication of good juvenile recruitment in those years. In 1978, however, catch of adult fish was high, and the group of young fish seen in 1975 and 1976 was less evident. Possibly, in Baptiste, adult fish were replaced by surviving older juveniles which had a relatively low catchability prior to the decline in adult population. The adult population in Baptiste was younger, on average, in 1978 than in 1975 or 1976, suggesting that young fish may have been recruited. Further observations on Baptiste are required to determine whether the results for 1978 contradict my hypothesis, or were merely fortuitous.

Johnson (1976) hypothesized that young fish recruited to the established population would grow rapidly to asymptotic size for the population. Historical patterns of growth and recruitment in my study lakes suggest that best average growth of a year-class occurs at the end of a period of good recruitment and during the first year of declining recruitment (Fig. 5). I interpret this to mean that recruitment is, initially, not growth dependent. After good recruitment of one or two year-classes, however, the recruited fish may begin to inhibit further recruitment, and only the fastest growing members of the next few year-classes are successful. The historical growth record on the scales, of course, reflects the growth of the successful members of the year-class, not the year-class as a whole. Finally, new recruits occupy all the available space, and further recruitment is restricted to replacement of natural mortality in the established gorup. New year-classes are thereafter suppressed, as described earlier.

The hypothesis that an established group of, mainly

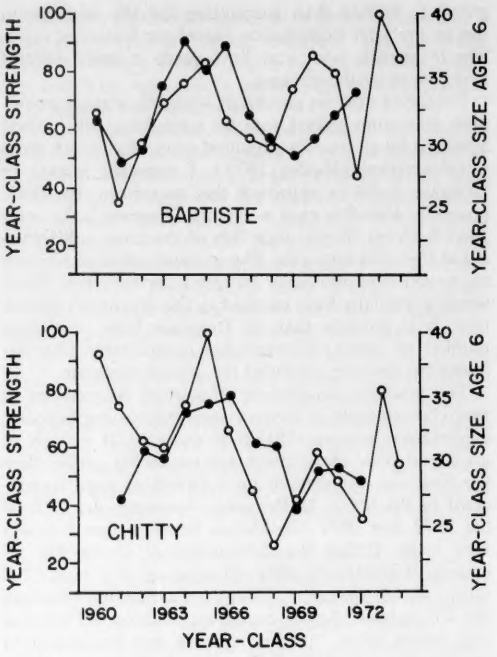

FIG. 5. Relative year-class strength for year-classes 1960–74 in Baptiste and Chitty lakes. The estimated strength of each year-class is shown as a percentage of the strongest year-class during the period (○). The back calculated length at age 6 of fish in year-classes 1960–72 is shown for comparison (●).

adult, whitefish can influence the survival and recruitment of their offspring in a way that regulates population size is consistent with a wide literature on the importance of behavior in regulating population size (Wynne-Edwards 1962). Although not without controversy, behavioral regulation of population size is generally accepted to exist among mammals, birds, and even insects (Krebs 1978). The importance of behavior in regulating population size is less well studied in fishes although the importance of territorial behavior for population regulation is recognized (Chapman 1962; Van den Assem 1967). In the case of whitefish the evidence, though not conclusive, indicates the likelihood of behavioral control of population size in unexploited populations. Furthermore, the responses of whitefish when exploited are logical consequences of the natural regulatory mechanisms (Johnson 1976) and I have hypothesized to occur. Rather than complicating the interpretation of population data (Power 1978) such hypotheses provide a unifying explanation for a wide range of empirical observations.

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Harvesting Strategies and Parameter Estimation for an Age-Structured Model[1]

R. B. DERISO

Curriculum in Marine Sciences, University of North Carolina, Chapel Hill, NC 27514, USA

DERISO, R. B. 1980. Harvesting strategies and parameter estimation for an age-structured model. Can. J. Fish. Aquat. Sci. 37: 268–282.

An age-structured model with knife-edge recruitment describes the dynamics of exploited, seasonally breeding populations. For management strategy, an equation is derived that characterizes the economic optimal state of exploitation of a modeled stock. A further generalization is derived to account for those species where recruitment to the mature stock occurs over many age-categories. Since the parameters all have phenomenological definitions, they can be estimated from information independent of the model. These parameters can also be estimated by regression of the model to catch and effort data. Test regressions of the general model are made on catch and effort data from three exploited fish stocks: the yellowtail flounder of New England, the Pacific halibut of Area II, and the haddock of Georges Bank; the corresponding R^2 values are 0.75, 0.87, and 0.86. Although the confidence intervals for individual parameter estimates are very large, the estimates compare favorably with published parameter values. Since only certain combinations of parameters from the general model appear to have low sensitivity to small perturbations to the data, some guiding suggestions are made that may lead to an improved robustness in the statistical procedures.

Key words: age-structured model, optimal harvesting strategy, spawner–recruit curves, parameter estimation

DERISO, R. B. 1980. Harvesting strategies and parameter estimation for an age-structured model. Can. J. Fish. Aquat. Sci. 37: 268–282.

Un modèle structuré par âge, avec recrutement bien tranché, décrit la dynamique de populations se reproduisant saisonnièrement et exploitées. En vue d'une stratégie de gestion, nous dérivons une équation caractérisant la condition optimale économique d'exploitation d'un stock modélisé. Une autre généralisation dérivée du modèle tient compte d'espèces chez lesquelles le recrutement au stock mature se produit sur plusieurs catégories d'âge. Possédant tous des définitions phénoménologiques, les paramètres peuvent être estimés à partir d'une information indépendante du modèle. Ils peuvent aussi être estimés par régression du modèle par rapport aux données de capture et d'effort. A titre d'essai du modèle général, nous établissons des régressions par rapport aux données de capture et d'effort chez trois stocks de poissons exploités : limande à queue jaune de Nouvelle-Angleterre, flétan du Pacifique de la zone II et aiglefin du banc Georges; les valeurs correspondantes de R^2 sont : 0,75, 0,87 et 0,86. Bien que les intervalles de confiance d'estimations de paramètres individuels soient très grands, les estimations se comparent favorablement aux valeurs publiées. Puisqu'il n'y a que certaines combinaisons de paramètres du modèle général qui semblent posséder une faible sensibilité à de petites perturbations des données, nous faisons certaines suggestions susceptibles d'améliorer les méthodes statistiques.

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STOCK–PRODUCTION models are traditionally based on the logistic equation or some similar function (e.g. Schaefer 1954; Pella and Tomlinson 1969; Fletcher 1978). As a class they have accumulated a long history of adverse criticism: their equation forms all imply continuous reproduction (as opposed to seasonal breeding), and in applying the models one must suppose that progeny age instantaneously to adulthood. And because of the composite nature of the terms in these models, the model parameters have no direct correspondence to observable phenomena. In consequence, one's estimates of model parameters cannot be corroborated by estimates independent of the model itself. But the traditional models do have an understandable appeal, despite their various invalidations. They are relatively free of mathematical complications, and for some stocks they fit catch–effort data fairly well.

A compromise between the traditional models (but

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overly simple) and a very realistic model biologically (but overly complex) would be desirable. In another paper (Deriso 1978), I propose such an alternative population model. It is a delay-difference model; it incorporates the effects of seasonal breeding and it approximates the behavior of a complete age-structured model.

The first part of this paper is devoted to a theoretical treatment of the delay-difference model. Discussed there are the separations of the adult survival parameter, the adult growth parameter, and the spawner–recruit relationship. Also treated is the influence of abiotic factors on recruitment. I then discuss the (economic) optimal exploitation of a stock, as modeled by the delay-difference equation, and I characterize the optimal equilibrium catch.

In the second part of this paper, I present a generalization of the delay-difference model. The generalized form will accommodate populations for which recruitment to the fishery occurs over many age categories (i.e. incomplete recruitment of young adults). The parameters of the model can be estimated by regression from catch and effort data; a review of pertinent statistical theory for time-series is presented.

Versions of the delay-difference model are then applied to New England yellowtail flounder, to Pacific halibut, and to Georges Bank haddock. Published estimates of certain parameters are compared to my regression estimates for those stocks. In most cases there is reasonable agreement between the two, but confidence intervals for individual parameters are very large.

The Delay-Difference Population Model

Summarized here are the assumptions needed for application of the delay-difference equation (as derived in Deriso 1978) for the case of an exploited seasonally breeding population. A form of the Brody weight equation is used to model the age-specific weights of the catchable adults in the population. The Brody equation is often used for this purpose (see Ricker 1975), and the difference form employed here is

$$(1) \qquad w_{i,t} = w_{k,t-(i-k)}\left\{\frac{1-\rho^{1+(i-k)}}{1-\rho}\right\}$$

where $w_{i,t}$ = average weight of a catchable adult (i years old) during the year t.

ρ = Brody's growth coefficient for weight; $0 \leq \rho \leq 1$. No units.

k = age of recruitment of individuals to the fishery.

The customary form of the Brody equation contains a correction term, of minor importance, which has been omitted from (1).

Catchable adults are assumed to experience a common annual mortality, which is an assumption shared with the Beverton-Holt year-class model. Hence, we can define l_t as the annual survival fraction of catchable adults from year t to year $t+1$. We must also assume that very few adults actually attain the maximum age that might be physiologically possible for the given stock. The latter assumption then permits us to extend the indexing of age categories in the model to infinity without inducing a significant error into the dynamics of the model population.

Now let $B_t = \sum_{i=k}^{\infty} w_{i,t} \cdot N_{i,t}$ be defined as the total biomass of the catchable population just prior to fishing in year t. Here $N_{i,t}$ is the number of individuals of age i. We can now construct a model that uses biomass measurements to estimate the parameters l_t and ρ, in contrast to standard procedures that require age-specific numbers of individuals in order to estimate l_t and that require age-specific weight of individuals in order to estimate ρ. The delay-difference model can be written as

$$(2) \qquad B_{t+1} = (1+\rho)l_t B_t - \rho l_t l_{t-1} B_{t-1} + w_{k,t+1} N_{k,t+1}$$

We assume here that recruitment $w_{k,t+1} \cdot N_{k,t+1}$ is a function of the biomass of spawners and that spawner biomass in year t is given by escapement $(B_t - C_t)$. The term C_t is the total catch (in weight) for year t, idealized to occur early in year t. We can write this spawner–recruitment relation as

$$(3) \qquad w_{k,t+1} N_{k,t+1} = F(B_{t+1-k} - C_{t+1-k})$$

If fishing occurs throughout the year, then spawning is assumed to occur at the end of the year, in which case the relationship (3) remains approximately valid as long as the natural mortality of adults remains constant and the total mortality of adults is not too large.

If an abiotic factor a_t influences recruitment survival, then spawner–recruit relation (3) can be made to depend on this abiotic variable. That is, we can write $w_{k,t+1} N_{k,t+1} = F(a_{t+1-k}, B_{t+1-k} - C_{t+1-k})$. If desired, a generalization of model (2) can be made that will accommodate density-dependent growth of adults. For example, we could make ρ a function of the abundance of adults, but growth generalizations for adults will not be pursued here.

The adult survival fraction l_t can be separated into natural survival and escapement from the fishery. Such a separation can be made when natural mortality is minimal during the period of harvesting; but, it can also be made by approximating l_t by the first-order term in its taylor series expansion about the value $l^*_t = 1$, which is a very accurate approximation when l_t is large in value. I have not explored the effects of errors from that approximation on the performance of our model. Let us make that separation of l_t and then we can write the delay-difference model as

$$(4) \qquad B_{t+1} = (1+\rho)lS_t - \rho l^2 \frac{S_t}{B_t} S_{t-1} + F(S_{t+1-k})$$

where l = annual natural survival fraction for catchable adults

$S_t = B_t - C_t$ = escapement of catchable adults (spawners) in year t. Units are in weight.

The model (4) contains explicit terms for growth of adults (ρ), natural survival of adults (l), escapement of adults (S_t), and a spawner–recruit relationship ($F(S_{t+1-k})$). Later in the paper, we will find separate estimates for all the parameters of (4) by the regression of (4) with catch and effort data. This separation of estimates is also possible for the generalized form of (4) that allows for incomplete recruitment.

Optimal Harvesting Strategies

We now turn to the problem of optimal harvest policies for the delay-difference model (4). If we are to optimize the harvesting of a resource over time, we cannot ignore the marketplace and concentrate solely on the maximization of biomass yield. In the optimization strategy, we must balance the current allocation of the resource against future economic benefits. Following Clark (1976a), let us assume that the objective of exploitation is maximization of the discounted present-value of net economic yield and that the net economic yield in year t can be expressed as

$$Y_t = \int_{Bt-Ct}^{Bt} \{p(x) - h(x)\}dx$$

where Y_t = net economic yield in year t from the harvest C_t that is taken from the adult biomass B_t. Units are in cash.

$p(x)$ = price per unit when the population's biomass is x.

$h(x)$ = harvest cost per unit weight when the population's biomass is x.

For example, if the unit price and unit harvest costs are constant (p,h constant) then $Y_t = (p - h)C_t$.

When a given cash payment Y_t is invested at compound interest, its value in year $t + n$ is given by $Y_t(1 + int)^n$ where int is the annual real rate of interest (usually about 2–4% per annum; cf. Clark 1976a). In year t, the discounted present-value PV_t of a sequence of catches for N many years is given by the formula

$$PV_t = \sum_{n=0}^{N} Y_{t+n}i^n$$

where i is equal to $1/1 + int$. The time horizon N is infinite for the analysis here.

To formulate the optimal harvesting problem for the model (4) we maximize PV_t, subject to the feasibility constraints $0 \leq C_{t+n} \leq B_{t+n}$ $(n = 0,1,2,\ldots)$. And by the method of Lagrange multipliers, as detailed in Appendix I, the optimal condition for equilibrium harvesting of the model population becomes

$$\tilde{Y}\{(1 + \rho)l - \rho l^2 \frac{S_e}{B_e}(1 + i) + i^{k-1}\dot{F}(S_e)\} = \frac{1}{i} - \rho\left(\frac{lS_e}{B_e}\right)^2$$

where $\tilde{Y} = \dfrac{\dfrac{\partial(Y_t)}{\partial B_t} + \dfrac{\partial(Y_t)}{\partial C_t}}{\dfrac{\partial(Y_t)}{\partial C_t}}$ and all partial derivatives are evaluated at an equilibrium population level.

S_e = equilibrium escapement (and spawners) for optimal harvest.

B_e = equilibrium biomass of the population prior to harvesting.

$\dot{F}(S_e)$ = derivative of F with respect to S_t, evaluated at equilibrium.

The condition (5) always characterizes the optimal equilibrium harvesting strategy, but certain convexity conditions (certainly satisfied in the case of a constant price p and a nonincreasing unit harvest cost $h(x)$) would be required to insure that a dynamic, nonequilibrium optimal strategy for harvesting, in time, would approach the equilibrium solution characterized by (5).

We can cast the optimal harvesting condition (5) into a form similar to the conventional form that applies to optimal harvesting in spawner–recruit curves. Let us use our previous example of p and h constant to illustrate a graphical representation of condition (5). Since we have assumed that $Y_t = (p - h)C_t$ for our example, it also follows that $\tilde{Y} = 1$. After algebraic manipulation of the condition (5), the following equation can be shown to be equivalent to the condition (5) for the case where $\tilde{Y} = 1$.

$$(6) \quad i^{k-1}rv\dot{F}(S_e) = 1 + rv\left\{(1 - i)\left(\frac{1}{i} - \rho l^2 \frac{S_e}{B_e}\right) - \rho l^2\left(1 - \frac{S_e}{B_e}\right)^2\right\}$$

where $rv = 1/(1 - \rho l)(1 - l)$ denotes the reproductive value (in units of biomass per unit biomass) of age k recruits in the virgin (unfinished) population. We can also interpret rv as the contribution of a unit biomass of recruitment, over its expected lifetime, to the biomass of the virgin population since fecundity is proportional to biomass in model (4).

In Fig. 1, a dome-shaped spawner–recruit curve has been applied to model (4) to provide a graphical interpretation of the optimal harvesting condition (6). Should adults be fully grown when caught at the S_e level for maximum sustainable yield ($i = 1$, $\rho = 0$), then we get the well-known relationship $rv\dot{F}(S_e) = 1$. However, if adults are growing when caught at the level of maximum sustainable yield, MSY, ($i = 1$, $\rho \neq 0$), then $rv\dot{F}(S_e) < 1$. And therefore, with the convex (dome-shaped) spawner–recruit curve, the escapement level S_e is increased beyond the S_e level of the first case where we had $\rho = 0$. When fully grown adults are fished at the optimal economic level of sustainable yield ($i \neq 0$, $\rho = 0$), then $rv\dot{F}(S_e) > 1$. And therefore, with a convex spawner–recruit curve, the escapement level decreases (from the S_e level where $i = 1$). Thus, whether the slope $rv\dot{F}(S_e)$ has a value larger or smaller than 1 will depend on the relative magnitudes of the interest and growth terms. The age of recruits, k, also affects the slope $rv\dot{F}(S_e)$ since the interest term i^{k-1} discounts future recruitment.

We cannot infer the optimal equilibrium catch for an

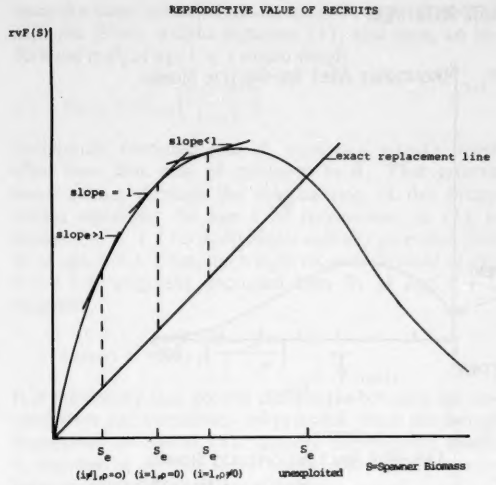

FIG. 1. Optimal escapement for a spawner–recruit curve. Reproductive value of total recruitment in an unexploited population is graphed versus spawners. Optimal escapement S_e is illustrated for three cases under the assumption that p and h are constant: maximum sustainable yield of fully grown adults ($i = 1$, $\rho = 0$) occurs with $rv\dot{F}(S_e) = 1$; maximum sustainable yield of growing adults ($i = 1$, $\rho \neq 0$) occurs with $rv\dot{F}(S_e) < 1$; optimal economic yield of fully-grown adults ($i \neq 1$, $\rho = 0$) occurs with $rv\dot{F}(S_e) > 1$.

iteroparous population directly from Fig. 1, but, from model (4), the following relationship can be derived

$$(7) \quad rvF(S_e) - S_e = rvC_e\left(1 - \rho l^2 \frac{S_e}{B_e}\right)$$

In contrast to the spawner–recruit analysis for semelparous populations, equation (7) shows that the difference $rvF(S_e) - S_e$ for iteroparous populations is not equal to C_e. The difference $rvF(S_e) - S_e$ is now equal to the reproductive value of a unit biomass of recruitment, corrected for growth, times the optimal catch C_e. For example, if adults are fully grown when caught ($\rho = 0$) then the catch C_e from an iteroparous population can be interpreted as though it were taken from recruits only; rvC_e would then be the total reproductive value of the harvested recruits if they were returned undamaged to the population.

We would also like to know the optimal harvesting strategy among all feasible harvesting strategies for model (4) not just the optimal equilibrium strategy. The version of model (4) for fully grown adults ($\rho = 0$) has also been discussed by Clark (1976b); from his analysis of this model, he suggests "... that for a general type of economic objective, the optimal harvest policy (among all feasible strategies) is in practise essentially determined in terms of a single optimal escapement level $x = x^*$ {$x^* = S_e$ in our notation here}, which is completely independent of the initial data. The theoretically optimal approach to equilibrium does depend on the initial data, but the dependence is minor and can probably be neglected in practise." We may also expect the optimal escapement level S_e to be applicable to optimal harvesting in the general model (4).

There are certain management advantages when a harvest policy is based on an optimal escapement level S_e. Random variations in recruitment survival or adult natural survival are automatically accounted for with such a harvest policy; and in a year of poor recruitment, the catch C_t would be reduced and the spawning stock $S_t = S_e$ would remain intact.

Estimation of S_e is a more difficult task. Parameter estimates from model (4) could be used to estimate S_e. Another approach to the estimation of S_e could be based on conservative estimation of the parameters in model (4) — by using, say, the estimated parameter values ± a standard error of the estimates (depending on the influence of the parameter on the magnitude of S_e) — so that an initially poor estimate of S_e would not endanger the stock. And once we had the conservative estimates, we might then use a generalization of the adaptive control approach (Walters and Hilborn, 1976) to refine the S_e estimates as more information became available.

The "Incomplete Recruitment" Model

Three basic patterns of recruitment can be distinguished (Ricker 1975): (i) knife-edge recruitment where all fish of a given age become vulnerable to the fishery, their vulnerability remaining constant throughout their lifetimes; (ii) platoon recruitment where the vulnerability of a year-class increases gradually with time, but where individuals, during any fishing season, are either fully catchable or not catchable; (iii) continuous recruitment where the vulnerability of a year-class gradually increases so that each fish, as it grows larger and older, experiences an increasing likelihood of being caught until a limit of maximum vulnerability is reached.

Model (4) expressly accommodates pattern (i), knife-edge recruitment, but the other patterns can be approximated by adjustment of the model. The latter two recruitment patterns are probably more common to fisheries. We can often treat continuous recruitment as though it were platoon recruitment (Ricker 1975).

With the single model displayed below, we can approximate recruitment patterns (ii) and (iii), both of which exhibit incomplete recruitment of age k fish to the fishery and spawning subpopulation (age k being the earliest age of significant vulnerability). Adult fish are now divided into two populations: an uncatchable population P_t of sexually immature fish and a catchable population B_t of sexually mature fish. The mature adults of any age are assumed to be larger in average size than the immatures, except for an overlap of sizes among those individuals most recently recruited. Of the immature adults P_t, a constant fraction $(1 - m)$ is re-

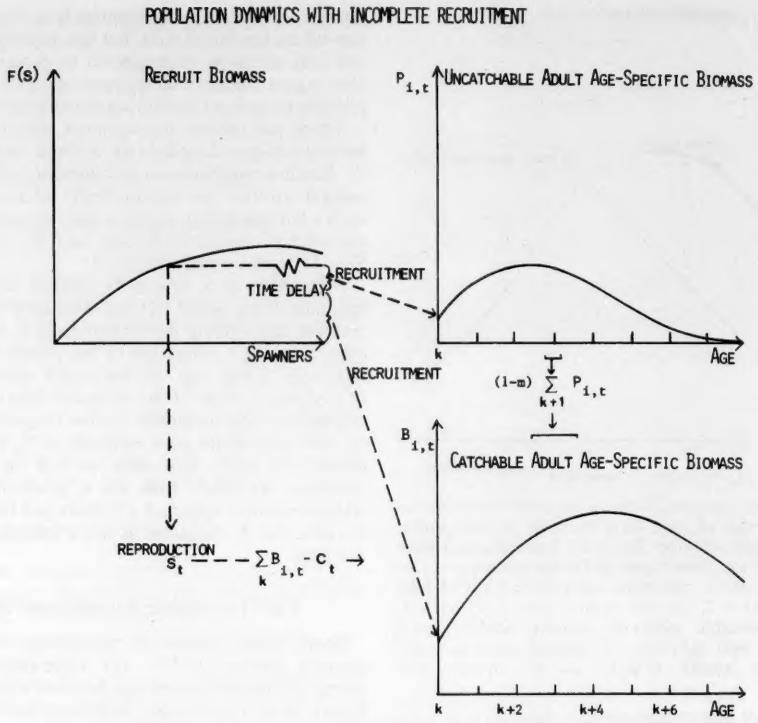

FIG. 2. A graphical representation of population dynamics with incomplete recruitment of young adults. Recruits enter both the uncatchable and catchable adult subpopulations. Spawners are those individuals in the catchable adult subpopulation that survive annual fishery exploitation.

cruited annually into the catchable population B_t. Such a recruitment pattern is somewhat idealized, since recruitment here coincides with the attainment of sexual maturity. It would be more accurate, of course, to use age-specific data on sex ratios, fecundity, and recruitment; however, such detailed information covering a large number of years is available only for a few fish stocks. Our purpose here is to develop a model that can be used with limited data and it is usually more reasonable to assume that the large individuals in B_t are responsible for spawning, rather than both small individuals in P_t and large individuals in B_t being equally responsible for spawning. With no natural mortality ($l = 1$), the equilibrium age-distribution of numbers of fish in P_t follows a negative exponential which is proportional to m^{i-k}. The "incomplete recruitment" model is derived in Appendix II; this model is given by the set of equations:

$$\begin{aligned} P_{t+1} &= (1+\rho)lmP_t - \rho(lm)^2P_{t-1} + F(S_{t+1-k}, a_{t+1-k}) \\ B_{t+1} &= (1+\rho)lS_t - \rho l^2 \frac{S_t}{B_t} S_{t-1} + (1-m)P_{t+1} \end{aligned} \tag{8}$$

where P_t = total biomass of the uncatchable adult population. Individuals in P_t are assumed to be sexually immature.

B_t = total biomass of the catchable adult population prior to harvesting in year t.

$S_t = B_t - C_t$; again C_t is the annual catch in year t, and S_t is the total spawner biomass in year t.

$1 - m$ = fraction of individuals in P_t that become catchable (enter B_t) at the beginning of year t (prior to harvesting in that year).

ρ = Brody's growth coefficient for weight.

The model (8) has been cast in a relatively simple form, and a diagram of the recruitment and survival processes in the model is shown by Fig. 2. The population dynamics underlying this model, however, are fairly complex. The weight equation used in model (8) for the catchables B_t accounts for much of the complexity.

Model (8) contains a single-step growth booster for the catchables so that catchable adults in any year-class may be made larger in size than the uncatchable adults

from the same cohort. Individuals in P_t grow in accord with the Brody weight equation (1), and thus, an individual in P_t of age $k + i$ would weigh

$$w_{k+i,t} = w_{k,t-i}\left(\frac{1 - \rho^{i+1}}{1 - \rho}\right).$$

Individuals recruited into B_t receive a growth boost after their first year of existence in B_t. That growth boost occurs through the reinitializing of the Brody weight equation; the age k of recruitment in (1) is changed to $k + i$ for individuals actually recruited into B_t at age $k + i$. Thus, the weight of an individual at age $k + i + j$, originally recruited into B_t at age $k + i$, becomes

$$w_{k+i+j,t} = w_{k+i,t-j}\left(\frac{1 - \rho^{j+1}}{1 - \rho}\right).$$

It is interesting that growth differences between the uncatchables and catchables are provided for in the simple framework of model (8); a more complicated model is required if in fact there are no growth differences between catchables and uncatchables.

The model (8) can be generalized along the lines of several arguments. The growth term ρ can be made a function of either abiotic or biotic variables. We can also assign to the uncatchable population a growth term that differs from the growth term for the catchable population. If young, immature fish were the principal harvest from a population (which is the situation for the Barents Sea/Bear Island cod stocks, (Garrod 1969)), then the catch C_t should be taken from P_t rather than from B_t. If the type of gear used by a fishery allows escapement of older, larger fish (or if, for some other reason, fish grow above a catchable size but yet many sexually mature fish actually get caught) then the model (8) should be generalized to account for such a circumstance; the gillnet fishery for Hudson River striped bass is a good case in point. In another paper (Deriso 1978) I presented a generalization of model (8) that will accommodate a stock made up of the three populations: young uncatchables, catchables, and old uncatchables.

Parameter Estimation for the General Delay-Difference Model (8)

The parameters of model (8) can be estimated from annual catches C_t and annual effort measures E_t. To recast the model into a form suitable for regression with catch–effort data, we first assume that the relationship between catch C_t and biomass value B_t (the biomass of the catchables just prior to harvesting) can be represented by the simple form

$$C_t = qE_tB_t$$

or, equivalently,

$$c_t = \frac{C_t}{E} = qB_t$$

where E_t = the annual measure of effort for year t, having units suitable to the techniques of the fishery, but standardized for all users of a single fish stock.

q = catchability coefficient, with dimensions of (unit effort)$^{-1}$.

c_t = catch per unit effort for year t, with dimensions of (weight per unit effort).

The biomass of the uncatchable part of the population P_t can not be measured directly from C_t and E_t data. In the second equation of (8), however, we see that P_t can be related to B_t. In fact, the set of equations (8) can be cast entirely in terms of the variables $B_t = c_t/q$ and C_t. By substituting the second equation of (8) into the first equation of (8) and by letting $B_t = c_t/q$, we get the following expression, which is equivalent to the general delay-difference model (8) but contains only observable quantities and parameters:

$$\begin{aligned}(9)\quad c_{t+1} = {} & (1 + \rho)l(1 - qE_t + m)c_t \\ & - l^2\{\rho(1 - qE_t)(1 - qE_{t-1}) \\ & + (1 + \rho)^2(1 - qE_{t-1})m + \rho m^2\}c_{t-1} \\ & + (1 + \rho)\rho l^3 m(1 - qE_{t-2})(1 - qE_{t-1} + m)c_{t-2} \\ & - (\rho l^2)^2 m^2(1 - qE_{t-2})(1 - qE_{t-3})c_{t-3} \\ & + q(1 - m)F\left\{(1 - qE_{t+1-k})\frac{c_{t+1-k}}{q}\right\}\end{aligned}$$

We have not yet specified the spawner–recruit (S–R) relationship $F(\cdot)$ for model (9). In Deriso (1978), however, a flexible three-parameter S–R curve has been developed that can be linked to the theoretical S–R relationships that arise when recruits are subject to prey limitation and intraspecific competition. The three-parameter S–R curve of Deriso (1978) is given by

$$(10)\quad R = F(S) = \frac{\alpha S}{(\beta S + 1)^{\gamma}}$$

where the constants α, β, and γ are all greater than (or equal to) zero; this S–R formulation contains the Beverton-Holt S–R curve ($\gamma = 1$) and the Ricker S–R curve ($\gamma \to \infty$, $\gamma\beta$ = constant) as special solutions. The three-parameter curve is employed in this paper for the S–R relationship of model (9).

We now turn to the problem of estimating the parameters of equation (9), the catch-and-effort version of model (8). We first review some pertinent aspects of large sample theory for a statistical time series having nonlinear parameters. If we assume that the explanatory variables in (9) (the c_t and E_t data on the right side of the equation) constitute a fixed known sequence, that the parameters in (9) are unknown, and that for each value of t the model (9) is valid up to an additive normally distributed $N(0, \sigma^2)$ random variable, then under fairly general conditions the least-square parameter estimates from a Gauss-Newton regression will tend asymptotically to a normal distribution around the "true" parameter values with variance proportional to σ^2; the residual mean square is a con-

sistent estimator for σ^2 (Fuller 1976, in particular Section 5.5). And in general, as Fuller emphasizes, with the proper interpretation of the information matrix all of linear regression theory will hold approximately for this type of nonlinear regression. One result of linear regression theory that we are interested in relates to the robustness of least-squares estimates: namely, that least-squares estimates are unbiased irrespective of the probability distribution of the independent, additive random variable in a regression equation (Chatterjee and Price 1977). Thus, the assumption of residuals as being normally distributed is not that important for obtaining unbiased estimates; however, it is extremely important for hypothesis testing, which I will not perform in this paper. To take a more realistic view of model (9), we must recognize that since the explanatory variables are generated by a stochastic mechanism, they can not be regarded as a strictly fixed and known sequence independent of "noise" in the system.

For parameter estimation in a stationary process (such as that for a population fluctuating around an equilibrium level), a least-squares statistic from a Gauss-Newton procedure can again be supported by theory. For example, Box and Jenkins (1970, particularly Chapter 7) state that "... for most situations, the maximum likelihood estimates are closely approximated by the least squares estimates ...", and they conclude that the limiting properties of maximum likelihood estimates can be extended to cover stationary time series. Again, to take a more realistic view of model (9), we must take into account the possibility that fishing effort may change in time and gradually move a population to a different equilibrium level. The least-square parameter estimates may still provide us with robust estimates of the "true" parameters because the model (9) explicitly accounts for effort E_t, but variance estimates are apt to be seriously biased. The technique of differencing variables in a time-series is discussed in Deriso (1978) as a method for transforming a nonstationary time-series into a stationary time-series, but I have not applied it to model (9).

Model (9), with the three-parameter *S–R* curve, contains a large number of state variables (the combinations of c_t and E_t), but it contains only seven parameters. Should the data for c_t and E_t be randomly distributed, and should the age of recruitment $k < 5$ years, we can in fact construct fourteen linearly independent combinations from the state variables of (9) (viz. c_t, c_tE_t, ...). When age of recruitment $k \geq 5$ years, we get sixteen combinations of the state variables. Thus, the dynamic behavior of model (9) is well constrained by the limited number of parameters it contains. It seems clear that least-squares estimates for the independent parameters of (9) will be fairly insensitive to random fluctuations in survival and growth of a population and to modest departures of a population from an equilibrium level. We shall see in the next section, however, that certain parameters in (9) appear to be statistically correlated.

Application of Model (8) to Three Fish Stocks

Test regressions of the "incomplete recruitment" model (8) were made with catch and effort data obtained for the following three fish stocks: yellowtail flounder of southern New England, haddock of Georges Bank, and Pacific halibut of area II. There are two reasons for selecting those fish stocks for application of model (8): they are iteroparous fish stock that are commercially important; other authors have analyzed age-specific data on these fish, covering close to the same years of data I will analyze, and thus they provide published results that I can compare to my results. In this paper, the results of the regressions of model (8) are summarized; the reader is referred to Deriso (1978) for a detailed presentation of those regression results.

Catch and effort data were obtained from the following three sources: flounder data[2] was obtained from Sissenwine et al. (1978); haddock data was obtained from Grosslein and Hennemuth (1973); halibut data was obtained from Ricker (1975). Reasonable values for the age of recruitment to the fishery, k, were found to be $k = 2$, $k = 2$, and $k = 5$, respectively, for the stocks flounder, haddock, and halibut (cf. the k values in references in Tables 1, 2, 3).

Equation (9) with the spawner–recruit curve (10) was parameterized by a "canned" nonlinear regression routine Stepit (Chandler 1973). The seven parameters in model (9, 10), as well as an additional temperature parameter for the flounder stock, were estimated by regressing catch and effort data (and temperature data for flounder). The temperature parameter, Temp, was included in the flounder regression because of the lack of convergence in regressions without that parameter and because Sissenwine (1974) found that recruitment of flounder is related statistically to temperature. Following Sissenwine (1977), the recruitment parameter, a, in (10) was modified for the flounder regression to $a_T = a(1 + \text{Temp}\ (T - 10))$, where T is Block Island average air temperature in °C (National Weather Service data), and where Temp is a slope parameter for measuring the effect of temperature variations (from the 10°C mean temperature, Years 1935–74) on recruitment biomass.

Results from the regressions of (9) have been encouraging. Generalized squared correlation coefficients R^2,

$$R^2 = 1 - \frac{\text{Residual sum of squares}}{\text{Total sum of squares around the mean}},$$

for the regressions to flounder, haddock, and halibut data are, respectively, the R^2 values 0.75, 0.86, and 0.87. Model predicted CPUE and observed CPUE for the three fish stocks are displayed in Fig. 3a, b, and c,

[2]1977 data for catch and effort are preliminary values; I have used a 1969 effort value that is 75% of the value given by Sissenwine et al. (1978) so that approximate survival from fishing $(1 - qE_t)$ remains ≥ 0.

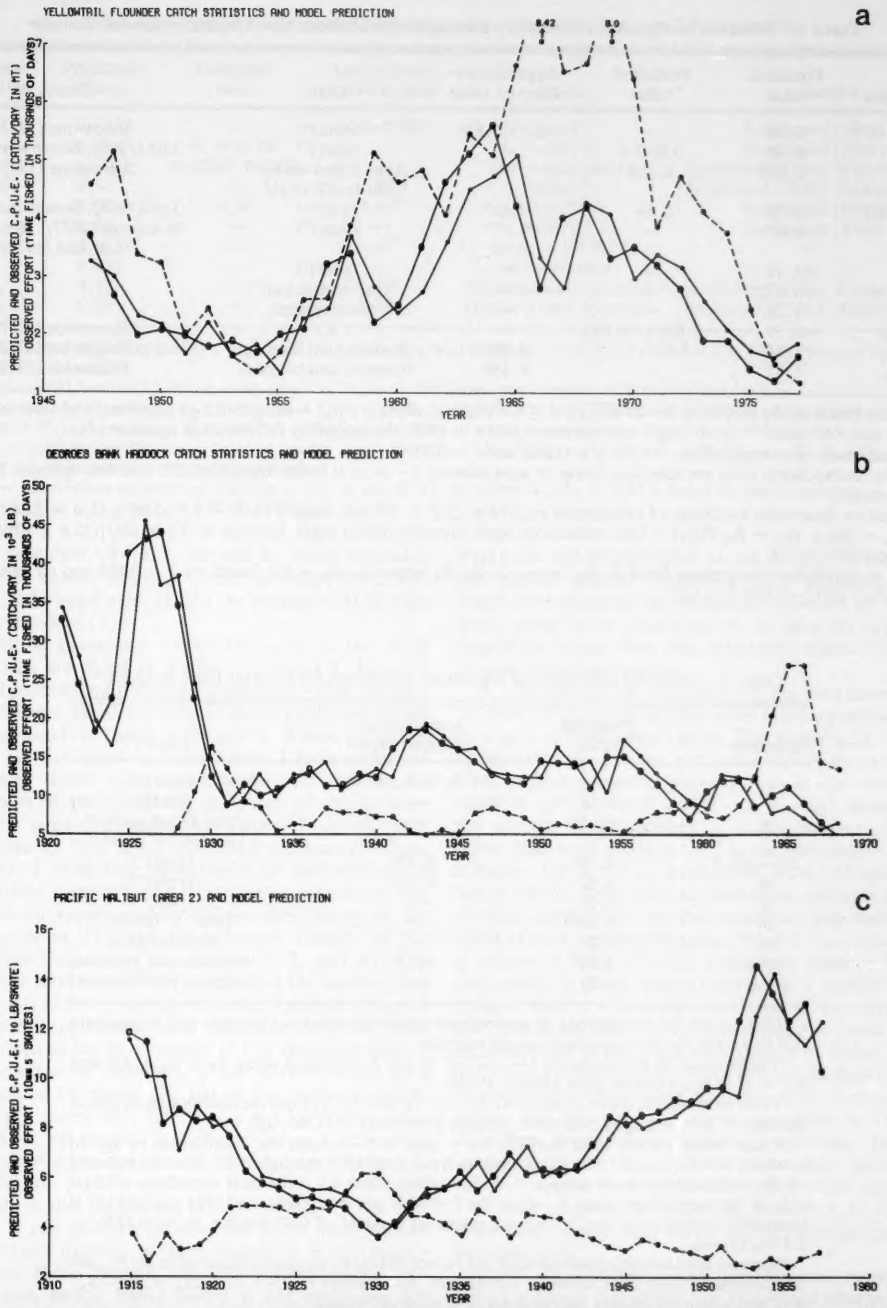
YELLOWTAIL FLOUNDER CATCH STATISTICS AND MODEL PREDICTION
a
PREDICTED AND OBSERVED C.P.U.E. (CATCH/DAY IN MT)
OBSERVED EFFORT (TIME FISHED IN THOUSANDS OF DAYS)
8.42
8.0
YEAR
GEORGES BANK HADDOCK CATCH STATISTICS AND MODEL PREDICTION
b
PREDICTED AND OBSERVED C.P.U.E. (CATCH/DAY IN 10³ LB)
OBSERVED EFFORT (TIME FISHED IN THOUSANDS OF DAYS)
YEAR
PACIFIC HALIBUT (AREA 2) AND MODEL PREDICTION
c
PREDICTED AND OBSERVED C.P.U.E.(10 LB/SKATE)
OBSERVED EFFORT (10ms SKATES)
YEAR

FIG. 3. Predicted CPUE (—●—), observed CPUE (—⊖—), and observed effort (—●—) are graphed versus year for three fish stocks: a) the yellowtail flounder of New England, b) haddock of Georges Bank, and c) Pacific halibut of Area II.

TABLE 1. Observed and predicted life-history parameters for southern New England yellow-tail flounder.

Parameter	Predicted value	Published value	Approximate observed value	Units	Source
ρ	0.80	—	0.57–0.82[a]	$(\text{year})^{-1}$	Sissenwine (1977)
l	0.38	0.8–0.9	—	$(\text{year})^{-1}$	Lux (1969); Sissenwine (1977)
q	0.018	0.168	—	$\left(\frac{\text{time fished each}}{\text{year in } 10^3 \text{ days}}\right)^{-1}$	Sissenwine (1977)
$l(1-q\bar{E})$	0.35	0.36	0.43[b]	$(\text{year})^{-1}$	Lux (1969); Sissenwine (1977)
m	0.47	—	0.5[c]	$(\text{year})^{-1}$	Sissenwine (1977); Hennemuth and Lux (1970)
α	15.39	—	—	$(\text{year})^{-1}$	
β	0.077	—	—	$(\text{year}\cdot\text{metric ton})^{-1}$	
γ	1.24	—	—	dimensionless	
Temp	−0.59	(−0.89)–(−0.68)	—	$(°\text{C})^{-1}$	Sissenwine (1977)
rv_A	1.64	—	2.3[d]	biomass/unit biomass	Sissenwine (1977)
rv_S	1.52	—	1.15[e]	biomass/unit biomass	Sissenwine (1977)

[a]Values based on the inequality $1-\sqrt{w_k/w_\infty} \leq \rho \leq 1-(w_k/w_\infty)$ where w_k/w_∞ = (length of 2-yr-olds/length of 10-yr-olds)$^{3.333}$ = (271 mm/460 mm)$^{3.333}$ from length measurements taken in 1943; the inequality follows from equation (1).

[b]Value based on average effort = 4.12, q = 0.168, and l = 0.85.

[c]Value extrapolated from net selection curves to approximate $1-m \simeq 0.5$, the fraction of 271 mm fish retained by a 4.5 inch codend.

[d]Based on Sissenwine's estimate of recruitment in 1974 as 22.8×10^6 fish, mean CPUE = 2.5×10^6 g/d, $q = 1.68 \times 10^{-4}$, and w_2 = 284 g. $rv_A = B_*/F(S_*)$ = total vulnerable equil. biomass/recruit equil. biomass $\simeq 2.5 \times 10^6/\{(22.8 \times 10^6)(284) \times (1.68 \times 10^{-4})\} = 2.3$.

[e]rv_S = equilibrium escapement fraction$\cdot rv_A = rv_A \times S_*/B_*$ where $S_*/B_* = 0.5$, based on $q = 0.168$ and on mean effort {viz. $0.5 = \exp(-0.168 \times 4.12)$}.

TABLE 2. Observed and predicted life-history parameters for Georges Bank haddock.[a]

Parameter	Predicted value	Approximate observed value	Units
ρ	0.85	0.75–0.94[b]	$(\text{year})^{-1}$
l	0.51	—	$(\text{year})^{-1}$
q	0.032	—	$\left(\frac{\text{time fished each}}{\text{year in } 10^3 \text{ days}}\right)^{-1}$
$l(1-q\bar{E})$	0.38[f]	0.47[c]	$(\text{year})^{-1}$
m	0.42	0.32[d]	$(\text{year})^{-1}$
α	3.12	—	$(\text{year})^{-1}$
β	0.0087	—	$(\text{year} \times 10^6 \text{ lb})^{-1}$
γ	1.2	—	dimensionless
rv_A	2.19	3.14[e]	(biomass/unit biomass)
rv_S	1.64	—	(biomass/unit biomass)

[a]Data source for calculations of approximate observed values is Grosslein and Hennemuth (1973); except for the asymptotic weight estimate.

[b]Values based on the inequality $1-\sqrt{w_k/w_\infty} \leq \rho \leq 1-(w_k/w_\infty)$ where $w_k = w_2$ = 2 lb and w_∞ = 33 lb (w_∞ estimate from Herald (1970)).

[c]Value based on the mean of ratios N_4/N_3, N_5/N_4, and N_6/N_5 from the distribution by age of numbers of fish, averaged over catch samples from years 1931 through 1965.

[d]Value based on the ratio $(0.47)N_2/N_3 = 0.68 = 1-m$ from the distribution by age of numbers of fish averaged over catch samples from years 1931 through 1965; calculation based on the rationale that catch sample $N_2 = (1-m)\hat{N}_2$, where $\hat{N}_2$ is the total abundance of 2-yr-olds in the population when $1-m$ of the 2-yr-olds are vulnerable, and that recruitment is essentially complete by age 3; thus an estimated survival of 0.47 implies $N_3 = 0.47\hat{N}_2 = 0.47N_2/(1-m)$.

[e]Since an approximate observed value for l is not available, an approximate value for rv_A has been estimated from the knife-edge recruitment model where $rv_A = 1/(1-\rho lS_*/B_*)(1-lS_*/B^*)$, and where parameters take midranges of observed values.

[f]Value based on mean effort = 7.8.

TABLE 3. Observed and predicted life-history parameters values for Area 2 Pacific halibut.

Parameter or function	Predicted value	Published value	Approximate observed value	Units	Source
ρ	0.64	—	0.65–0.88[a]	(year)$^{-1}$	Southward (1968)
l	0.73	0.79–0.86		(year)$^{-1}$	Southward (1968)
q	0.0033	0.00907–0.0118	—	(10^4 skates/year)$^{-1}$	Southward (1968); Ricker (1973)
$l(1-q\bar{E})$	0.64	—	0.56[b]	(year)$^{-1}$	Southward (1968); Ricker (1973)
m	0.001	0.95	0.68[c]	(year)$^{-1}$	Southward (1968)
α	1.86	—	—	(year)$^{-1}$	Southward (1968)
β	0.0033	—	—	(year $\times$ 10^6 lb)$^{-1}$	—
γ	1.011	—	—	dimensionless	—
rv_A	4.68	—	4.87[d]	(biomass/unit biomass)	Southward (1968); Ricker (1973)
rv_S	4.09	—	3.28[e]	(biomass/unit biomass)	Southward (1968); Ricker (1973)

[a]Values based on the inequality $1-\sqrt{w_k/w_\infty} \leq \rho \leq 1-(w_k/w_\infty)$ where $w_k = w_5 \simeq 15$ lb and $w_\infty \simeq 120$ lb (average weight of 20-yr-olds in the catch).

[b]Total annual survival based on mean effort = 38.01 $\times$ 10^4 skates/year, and on midrange q and l values.

[c]Value based on Southward's estimate that 85% of 9-yr-old halibut are fully vulnerable to fishing; approximate ratio of 9-yr-olds in P_t to 9-yr-olds in B_t is $m^{(9-5+1)} = 0.15$; $m \simeq 0.68$.

[d]rv_A = total vulnerable equil. biomass/recruit equil. biomass = $B_*/F(S_*) = (1-m)/\{(1-\rho lS_*/B_*)(1-lS_*/B_*)(1-\rho lm) \times (1-lm)\}$ where parameters take midrange values.

[e]rv_S = equilibrium escapement fraction $\times$ rv_A = $rv_A \times S_*/B_*$ where $S_*/B_* = 0.67$ is based on the midrange $q = 0.0104$ and mean effort $0.67 = \exp(-0.0104 \times 38.01)$.

where predicted CPUE is derived by using estimated parameter values, observed effort, and observed CPUE in the right-hand side of (9) to predict CPUE (the left-hand side of (9)).

Predicted parameter values for each of the three fish stocks are listed in Tables 1, 2, and 3. In some cases, I have found published parameter values to compare with the predicted values; those published values are also listed in Tables 1, 2, and 3. Where published values are not listed in those tables, I have estimated parameter values from supplementary information independent of the model for purposes of comparisons with the predicted parameter values. I should have been able to find more published parameter values, however, I took this opportunity to demonstrate in those tables a procedure for parameter estimation that is based on supplementary information; many of the parameters in (9) can be estimated outside of the regression framework (see Tables 1, 2, and 3). The values for total survival $l(1-q\bar{E})$ and for reproductive values, rv_A and rv_S, are also listed in Tables 1, 2, and 3 since they apparently have low sensitivity to small fluctuations in the fit of model (9) to observed data.

Standard error estimates, which were obtained by a linearization of model (9, 10) in the program Stepit, have been unreliable. But this is not surprising since the matrix of second partial derivatives (derivatives of the error mean square with respect to parameters in (9)) is not positive definite. However, all diagonal elements in that matrix are positive definite. Since that matrix is not positive definite, the error mean square surface (as a function of the parameters) is not roughly quadratic (bowl-shaped) near the final parameter estimates and, hence, linear theory is not applicable for obtaining standard error estimates (that is, standard errors are not proportional to the inverse of that second partial matrix). Standard error estimates from Stepit have exceeded the parameter values in all regressions; those error estimates are at least an order of magnitude larger than the parameter values obtained in most of the regressions.

A measure of the variability of the parameters can be obtained by sampling the error mean square surface on a grid of parameter values. The quantity Q, (mean square error at arbitrary parameter values/mean square error at final parameter values) $-$ 1, is approximately equal to $(p/(n-p))F_p(p, n-p, 1-\alpha_0)$ where p is the number of parameters, n is the number of data points that were predicted, F_p is the cumulative F distribution for a $1-\alpha_0$ probability level (Draper and Smith 1966). Approximate confidence intervals for individual parameters are the minimum and maximum value of each parameter along a fixed $1-\alpha_0$ contour of Q values. A joint $(1-\alpha_0)$ confidence contour for all parameters is given approximately by a contour of Q along a fixed $1-\alpha_0$ value. In Table 4, the minimum and maximum value of each parameter is listed based on samples of Q that were encountered by Stepit as the minimization routine zig-zagged toward the final parameter estimates. Since Stepit did not sample all parameter values, the minimum and maximum values in Table 4 constitute lower-bound (interior) estimates for the confidence intervals; in some cases only parameter values above a final parameter value were sampled. The main purpose for including Table 4 is to demonstrate that there is large variability in individual parameter estimates.

Stepit also computed an approximate correlation matrix for each regression. We should not interpret that matrix as being completely accurate, however, because

TABLE 4. Minimum estimates of 95% confidence intervals for parameters in (9).

Parameter	Minimum confidence intervals for flounder regression	Minimum confidence intervals for haddock regression	Minimum confidence intervals for halibut regression
ρ	0.38–1.0	0.71–0.98	0.001–0.9
l	0.38–0.75	0.49–0.57	0.73–1.0
q	0.0073–0.0426	0.005–0.033	0.001–0.008
m	0.0913–0.51	0.32–0.42	0.001–0.5
α	2.32–15.4	0.11–3.12	0.4–1.86
β	0.077–0.475	0.009–1.0	0.003–0.005
γ	0.66–1.24	0.001–3.1	0.5–1.31
Temp	(−0.92)–(−0.49)		

of the problems stated earlier about the mean square error surface. In all regressions, however, that matrix does indicate relatively large correlations among certain parameters in (9): ρ and l appear to be negatively correlated; α, β, and γ appear to be highly correlated; l and q appear to be negatively correlated; m and q appear to be highly correlated; β and q appear to be negatively correlated. We shall return to a discussion of these correlations later in this paper.

An important test for any regression on time series data is a test for systematic errors in the residuals (predicted minus observed CPUE). Perhaps the easiest test to apply is a large sample, turning point test (Kendall 1973), where the sum of the number of peaks (residual value greater than adjacent values) and the number of troughs (residual value less than adjacent values) is tested against the expected number of turning points $\{\frac{1}{2}-(n-2)\}$ in the normal distribution with a standard derivation given by $\sqrt{(16n-29)\div 90}$, where the expected value is based on the assumption that residuals are randomly distributed. The observed number of turning points for the flounder, haddock, and halibut regressions are, respectively, the values 19, 32, and 29; these observed values are very close to their corresponding expected values of 19.33, 30.66, and 27.33. All three regressions easily pass the turning point test for any reasonable level of significance (e.g. $\alpha_0 = 0.05$). Graphical plots of residuals versus predicted CPUE were also made for each of the three data sets. Inspection of those plots by eye revealed no trend in the plots and points appear to be randomly distributed with a concentration of points lying within a horizontal band (as one would expect for residuals distributed normally).

Fishing mortality corresponding to maximum sustainable yield was also computed for the three fish stocks. Formula (6) can easily be shown to be valid for MSY of populations modeled by the incomplete recruitment model (except rv on the left-hand side of (6) now pertains to the incomplete recruitment model, whereas rv on the right-hand side of (6) is computed according to the equation given below (6)). Since the parameter q acts as a scaling parameter and since q estimates are low in value, compared to published values, MSY estimates, C_e, are apparently scaled too high in value. Estimates of population biomass, B_e, are apparently scaled too high in value, also. Fishing Mortality fractions calculated by the ratio C_e/B_e appear more reliable, since the scaling error is cancelled in such a ratio.

Short-term and long-term MSY mortality fractions were predicted. The equilibrium biomass B^* was calculated for each of the three fish stocks and it represents predicted equilibrium levels for those stocks during the years in our nonlinear regressions; it is based on predicted parameter values in the tables in this paper and on average fishing effort during those years of regression. The B^* numbers are less in value than B_e for each of the three fish stocks, where B_e is the equilibrium biomass that is predicted to occur when the population has been fished at MSY for a long period of time. The removal of MSY from those three stocks is predicted to result in a gradual transition from a biomass level B^* to a lower biomass level B_e. Initial removal of MSY from those stocks is predicted to occur by applying the short-term MSY fishing mortality fraction, C_e/B^*, to those stocks. As equilibrium biomass is reduced, continued removal of MSY is predicted to occur by gradually increasing fishing to the long-term MSY fishing mortality fraction C_e/B_e.

Short-term MSY fishing mortalities for the flounder, haddock, and halibut stocks are respectively the predicted fractions 0.41, 0.28, and 0.15; long-term MSY fishing mortalities for these stocks are, respectively, the predicted fractions 0.7, 0.42, and 0.29. Predicted short-term and long-term MSY escapement fractions can be computed as being equal to (1-fishing mortality) and by taking natural logarithms of the inverse of these MSY escapement fractions, MSY fishing mortality rates can be calculated; short-term MSY fishing mortality rates for the flounder, haddock, and halibut stocks are, respectively, the predicted values 0.53, 0.33, and 0.16; the corresponding long-term MSY fishing mortality rates are 1.2, 0.55, and 0.35. The mortality rates are more suitable for comparison with published quantities than are the mortality fractions.

Predicted MSY fishing mortality rates were compared with published rates for the three fish stocks. The

short-term MSY fishing mortality rates are smaller than the published rates and long-term predictions are larger than published rates. Sissenwine (1977) suggests that a reduction in fishing effort on the flounder stock would have caused an increase in catch during the 1943–65 time period; a fishing mortality rate of 0.69 is indicated by the published q value in table 1 (based on mean effort = 4.12) and thus the short-term optimal rate of 0.53 agrees qualitatively with his suggestion. However, model (9) predicts that the removal of C_e from the flounder stock will lead in time to a MSY fishing mortality rate of 1.2. Grosslein and Hennemuth (1973) suggest that a fishing mortality rate of 0.47 for the haddock stock is a reasonable value for MSY catch; their predicted rate is larger than the short-term MSY fishing mortality rate of 0.33. However, model (9) again predicts that the removal of C_e from the haddock stock will lead in time to a MSY fishing mortality rate of 0.55. The comparison of predicted against published MSY fishing mortality rate for halibut is more difficult. Southward (1968) estimated an MSY fishing mortality rate of 0.4 for age 8–20 halibut, however, since the predicted MSY fishing mortality rate is based on an age of 5 for recruitment, we should spread Southward's estimated rate over three additional age-classes for purposes of comparison. A simple method to accomplish that spread is to equate expected lift-time fishing survivals:

$$\exp(-0.4(13)) = \exp(-F(16))$$

and solve for F to get $F = 0.33$. That adjusted MSY fishing mortality rate ($F = 0.33$) is larger than the short-term predicted MSY rate of 0.16 and it is smaller than the long-term predicted fishing rate of 0.35.

One-year forecasts were made for the halibut stock from the period 1958–75, which are based on CPUE data not used in the regression given earlier in this paper. That forecast is shown in Fig. 4 and it represents predicted CPUE for each year, say $t + 1$, based on observed CPUE and effort data for years prior to year $t + 1$ and on model (9) with the predicted parameter values in Table 3. In 1967, I.P.H.C. Area 1 data was incorporated as part of Area 2 data (Skud 1977) and the forecast in Fig. 4 is based on those combined data (given in Myhre et al. 1977). Prior to 1967, Area 1 halibut catch was less than 2% of Area 2 halibut catch and, thus, by combining data we should not introduce serious errors in those forecasts. The 1-yr forecasts are reasonable with a generalized $R^2 = 0.71$; however, those forecasts are especially poor during the period of 1972–75, which suggests that some changes may have occurred in the fishery or in the stock in recent years. In fact changes have occurred in recent years with the increased exploitation of undersized halibut. Catches of halibut were in a decline from 1958 to 1975 despite the low directed fishing effort and that is contrary to the long-term forecast based on MSY at $F = 0.35$ (using midpoint of published $q = 0.01$, MSY at effort = 3.4×10^5 skates/year).

Estimated spawner-recruit curves for the three fish stocks are all slightly convex (i.e. $\gamma > 1$), however the halibut S–R curve is nearly identical to a Beverton-Holt S–R curve. I have visually inspected values of estimated recruitment that were used internally in the regressions for estimating α, β, and γ; there is a large amount of variability in those estimated recruit values from recruit values that are predicted by the S–R curves. Since estimated spawner values were generally spread over only a small portion of the spawner axis, α, β, and γ estimates represent values that approximate a S–R curve over the data range only; this should help explain the correlations between these parameters.

Discussion of Regression Results

In all regressions of model (8), there has been a tendency for fishing mortality to be underestimated and natural mortality to be overestimated in comparison to published values, however total annual survival estimates have been favorable. The low estimated catchability coefficients is probably due, in part, to the approximation of fishing survival as a linear function of effort (i.e. by assuming that annual fishing survival $\simeq 1 - qE$). It is a straight-forward exercise to modify (9) to a form in which fishing survival depends on effort through the exponential function. However, CPUE would not then be proportional to B_t and the equation which results from that modification would have much more nonlinearity in its parameters than those in (9).

The correlation between estimates for the growth parameter ρ and l the survival parameter suggests that Stepit had difficulty in deciding (statistically) whether the data consisted of a large number of small fish or whether a few large fish were present in the data, however quantities that depend primarily on biomass are predicted with more precision (e.g. reproductive value). We should be able to improve both the ρ and l estimates by taking a Bayesian approach to the estimation of ρ: age-specific weight estimates are usually known apriori, and a one-step Brody weight equation could then be fitted to the weight estimates. A prior ρ estimate and corresponding variance estimate could then be used in regressions of (9). The Bayesian approach to parameter estimation could also improve the estimates for other parameters in (9, 10) when auxiliary information (e.g. recruitment year-class abundance) is available.

Trend in the data was not removed prior to regression. It is likely that a portion of the parameter confidence intervals in Table 4 were used primarily to estimate trend in the data; such parameter values would not be indicative of values within "true" confidence intervals. Techniques are available for removing trend in data (Box and Jenkins 1970) and this should be a fruitful direction for further research.

The regressions of model (9) have provided a starting point for further research on applications of the model. We have seen that all parameters in that age-

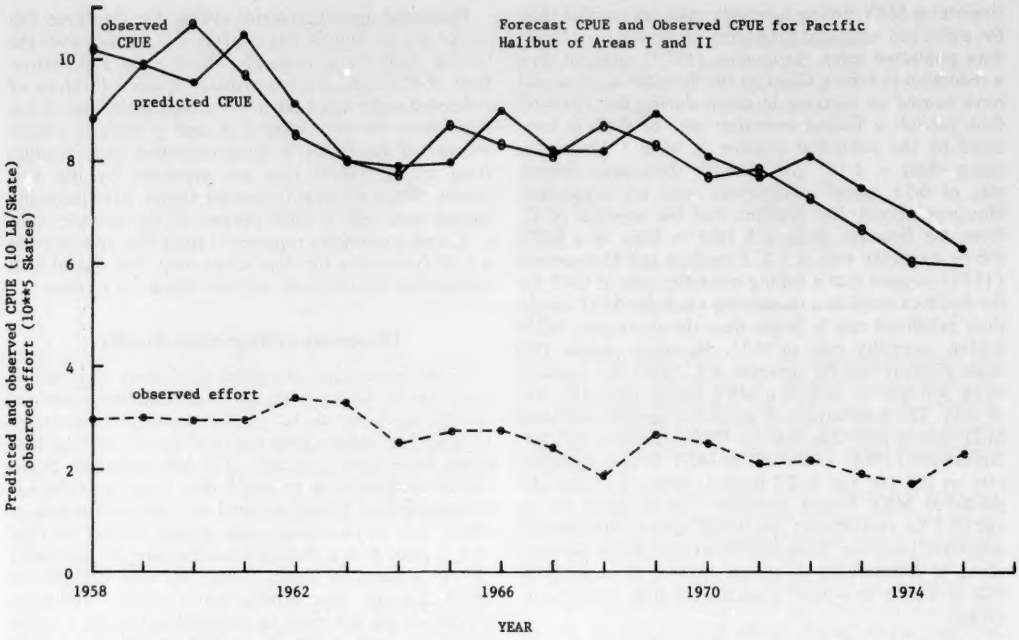

FIG. 4. Forecasted CPUE, observed CPUE, and observed effort are graphed versus year for Pacific halibut of Areas I and II.

structured model can be estimated from CPUE and effort data, that the parameters can also be estimated with information from independent biological studies, and that there is usually reasonable agreement between the two types of parameter estimates, except for estimates of q. The regressions have properly ranked reproductive value for the three fish stocks: the short-lived flounder has predicted rv_A and rv_S values that are smaller than the corresponding predicted haddock rv_A and rv_S values, whereas the long-lived halibut has predicted rv_A and rv_S values that are correspondingly larger than both flounder and haddock values. The regressions have predicted short-term and long-term MSY escapement fractions that seem reasonable when compared with published information; however, recent data on halibut suggest that MSY prediction may be inaccurate. Those predicted escapements accord with our intuition in that predicted halibut escapement is largest (for a long-lived fish) followed by the smaller haddock escapement, and the flounder escapement is smaller than both haddock and halibut values. As we have attempted to extract more detailed information about individual processes (e.g. growth or survival), accuracy of parameter estimates has been reduced; large correlations are present between many of the parameters in (9). From a statistical point of view, the use of auxiliary information and the removal of trend in data should greatly improve the precision of parameter estimates.

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Appendix I

The method of Lagrange multipliers may be used to derive formally the optimal equilibrium harvesting condition (5). Consider the Lagrange expression

$$L = \sum_{t=0}^{\infty}\Big\{i^t Y_t(B_t, C_t) - \lambda_t\Big(B_{t+1} - (\rho+1)(B_t - C_t) + \rho I^2(B_{t-1} - C_{t-1})\Big(1 - \frac{C_t}{B_t}\Big) - F(B_{t+1-k} - C_{t+1-k})\Big)\Big\}$$

For an interior solution, since boundary constraints do not apply, we have the necessary conditions

$$\frac{\partial L}{\partial B_t} = 0 \quad (t \geq 1); \qquad \frac{\partial L}{\partial C_t} = 0 \quad (t \geq 0).$$

Therefore,

$$\text{(a)}\quad \frac{\partial L}{\partial B_t} = i^t Y_{B_t} - \lambda_t(-(\rho+1)I + \rho I^2(B_{t-1} - C_{t-1})C_t B_t^{-2}) - \lambda_{t+1}\rho I^2\Big(1 - \frac{C_{t+1}}{B_{t+1}}\Big) + \lambda_{t+k-1}\dot{F} - \lambda_{t-1} = 0; \quad t \geq 1$$

and

$$\text{(b)}\quad \frac{\partial L}{\partial C_t} = i^t Y_{C_t} - \lambda_t\Big((\rho+1)I - \rho I^2\Big(\frac{B_{t-1} - C_{t-1}}{B_t}\Big)\Big) + \lambda_{t+1}\rho I^2\Big(1 - \frac{C_{t+1}}{B_{t+1}}\Big) - \lambda_{t+k-1}\dot{F} = 0; \quad t \geq 0$$

where

$$Y_{B_t} = \frac{\partial Y_t}{\partial B_t} \text{ and } Y_{C_t} = \frac{\partial Y_t}{\partial C_t}$$

By adding and rearranging (a) and (b), we get

$$\text{(c)}\quad i^t(Y_{B\,t} + Y_{Ct}) + \lambda_t\Big(\frac{B_t - C_t}{B_t}\Big)\rho I^2\Big(\frac{B_{t-1} - C_{t-1}}{B_t}\Big) = \lambda_{t-1}.$$

If we assume that we have an equilibrium solution B_e, with catch C_e, then

$$\text{(d)}\quad i^t(Y_B + Y_C) + \lambda_t\rho\Big(\frac{IS_e}{B_e}^2\Big) = \lambda_{t-1}, \quad t \geq 1$$

where $S_e = B_e - C_e$.

After multiplying (b) by $\rho\Big(\frac{IS_e}{B_e}\Big)^2$ and substituting (d) we get

$$i^t Y_C\rho\Big(\frac{IS_e}{B_e}\Big)^2 - (\lambda_{t-1} - i^t(Y_B + Y_C))A + (\lambda_t - i^{t+1}(Y_B + Y_C))\rho I^2\frac{S_e}{B_e} - (\lambda_{t+k-2} - i^{t+k-1}(Y_C + Y_B))\dot{F} = 0$$

where $A = (1+\rho)I - \rho I^2\dfrac{S_e}{B_e}$.

Expression (b) for year $t-1$ is given by

$$i^{t-1}(Y_C) - \lambda_{t-1}A + \lambda_t\rho I^2\frac{S_e}{B_e} - \lambda_{t+k-2}\dot{F} = 0,$$

and the difference of the last two expressions becomes

$$\text{(e)}\quad i^{t-1}Y_C\Big(i\rho\Big(\frac{IS_e}{B_e}\Big)^2 - 1\Big) + i^t(Y_B + Y_C)A - i^{t+1}(Y_B + Y_C)\rho I^2\frac{S_e}{B_e} + i^{t+k-1}(Y_B + Y_C)\dot{F} = 0.$$

By rearranging (e) we get the optimal equilibrium condition (5):

$$\frac{Y_B + Y_C}{Y_C}\left\{(\rho + 1)l - \rho l^2 \frac{S_e}{B_e}(1 + i) + i^{k-1}\dot{F}(S_e)\right\} = \frac{1}{i} - \rho\left(\frac{lS_e}{B_e}\right)^2.$$

Appendix II

In this appendix, I derive the "incomplete recruitment" version of model (2). For a population of mixed ages, let us assume that individuals of ages k and older are annually recruited into a fishable subpopulation. During each year, only the fraction $1 - m$ of the aged k year-class individuals are recruited into the fishable subpopulation, and each year a fraction $1 - m$ of surviving individuals (of ages $> k$ and not previously recruited) are also recruited into the fishable subpopulation. Let P_t represent the total biomass of individuals (aged k or older at the beginning of year t) that have not been recruited to the fishable population prior to year t.

We can write an age-structured model for P_t that is analogous to model (2):

(a) $P_{t+1} = (1 + \rho)lmP_t - \rho(lm)^2P_{t-1} + w_{k,t+1}N_{k,t+1}$

where m is analogous to a survival term since m is the annual fraction of individuals that remain uncatchable.

At the beginning of each year $t + 1$, $(1 - m)P_{t+1}$ is the total biomass of individuals that are recruited to the fishable population in that year. Let B_{t+1} represent the total biomass of individuals (aged k or older at the beginning of year $t + 1$) that are vulnerable to a fishery in year $t + 1$; note that B_{t+1} includes the biomass of new recruits $(1 - m)P_{t+1}$. I shall now shows that the single-step Brody weight equation (1) along with the usual assumption of a common survival fraction for adults leads to the following age-structured model for individuals in B_t,

(b) $B_{t+1} = (1 + \rho)l_tB_t - \rho l_tl_{t-1}B_{t-1} + (1 - m)P_{t+1}$

We can write B_{t+1} as the sum of surviving individuals that have been recruited from P_{t+1}, P_t, P_{t-1}, . . .:

(c) $B_{t+1} = (1 - m)\sum_{j=0}^{\infty}\sum_{i=0}^{\infty} w_{k+i}N_{k+i,t+1-j}\left(\frac{1 - \rho^{j+1}}{1 - \rho}\right) \times \prod_{h=1}^{j} l_{t+1-h}$

where $\sum_{i=0}^{\infty} w_{k+i}N_{k+i,t+1-j} = P_{t+1-j}$,

$\left(\frac{1 - \rho^{j+1}}{1 - \rho}\right)$ represents additional growth for individuals recruited into B_{t+1} in year $t + 1 - j$, and $\prod_{h=1}^{j} l_{t+1-h}$ represents the fraction of individuals (recruited into B_{t+1} in year $t + 1 - j$) that survive to year $t + 1$.

By writing an analogous expression to (c) for B_t, by multiplying B_t by l_t, and by performing simple algebra, we see that

$$B_{t+1} - l_tB_t = (1 - m)\sum_{j=1}^{\infty}\sum_{i=1}^{\infty} w_{k+i}N_{k+i,t+1-j}\rho^j\prod_{h=1}^{j} l_{t+1-h} + (1 - m)P_{t+1}$$

or, equivalently,

(d) $B_{t+1} - l_tB_t = (1 - m)\sum_{j=1}^{\infty} P_{t+1-j}\prod_{h=1}^{j} l_{t+1-h} + (1 - m)P_{t+1}$

By considering expression (d) for $B_t - l_{t-1}B_{t-1}$, by multiplying that expression by ρl_t, and by performing simple algebra, we see that

(e) $\rho l_t(B_t - \rho l_{t-1}B_{t-1}) = (1 - m)\sum_{j=1}^{\infty} P_{t+1-j}\prod_{h=1}^{j} \rho l_{t+1-h}.$

By subtracting expression (e) from expression (d), we get the model (b); the proof is complete.

Sex Determination of the Ommastrephid Squid *Illex illecebrosus* Using Beak Morphometrics

M. C. MERCER[1], R. K. MISRA, AND G. V. HURLEY

Department of Fisheries and Oceans, Research and Resource Services, St. John's, Nfld. A1C 5X1

MERCER, M. C., R. K. MISRA, AND G. V. HURLEY. 1980. Sex determination of the ommastrephid squid *Illex illecebrosus* using beak morphometrics. Can. J. Fish. Aquat. Sci. 37: 283–286.

The beak morphometrics of the ommastrephid squid *Illex illecebrosus* were analyzed as possible criteria for determining sex composition of beak samples taken from predator stomachs. A multivariate analytical procedure was adopted to elucidate the course of discriminant analysis needed in this study. The discriminant functions presented here can be employed usefully in identifying the sex of an unknown individual from either the upper or lower beak morphometrics.

Key words: squid, *Illex*, beak, morphometrics, multivariate, discriminant analysis, sex determination

MERCER, M. C., R. K. MISRA, AND G. V. HURLEY. 1980. Sex determination of the ommastrephid squid *Illex illecebrosus* using beak morphometrics. Can. J. Fish. Aquat. Sci. 37: 283–286.

La morphométrie des mâchoires de l'encornet ommastrephide, *Illex illecebrosus*, a été analysée comme critère possible de détermination de la composition par sexe d'échantillons de mâchoires prélevés dans les estomacs de prédateurs. Nous avons adopté une procédure analytique à variables multiples pour préciser le cours de l'analyse discriminante requise dans cette étude. Les fonctions discriminantes présentées ici peuvent être utiles dans l'identification du sexe d'un subjet inconnu d'après la morphométrie soit de la mâchoire supérieure, soit de la mâchoire inférieure.

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THE ommastrephid squid *Illex illecebrosus*, a summer migrant to inshore Newfoundland waters, is an important prey item for many species of whales (Sergeant 1962; Sergeant and Fisher 1957; Squires 1957), seabirds (Ennis and Collins 1978), and for many species of fish (Vinogradov 1972; Butler 1971; Squires 1957; Templeman 1944). Like other cephalopod species its soft body is quickly digested and often the only remains to be found in predator stomachs are the chitinous beaks and on occasion the pens (Draqovich and Wise 1969).

Cephalopod beaks from predator stomachs have been used to give information on relative abundance of families in different oceanic regions (Clarke 1978), as criteria for species and sometimes even stock identification (Clarke 1962a), and as a technique to study whale migration (Clarke 1972). None of the previous studies however has attempted to use beak morphometrics as a means of sex determination.

Such a technique would enable estimates to be made of the proportion of each sex found in predator stomachs which might have applications, for example, in helping to explain the areal and seasonal differences in sex ratios that have been noted in squid populations found in Newfoundland waters (Mercer 1973). To this end the present study of squid beak morphometrics, using multivariate statistical procedures, was carried out.

Materials and methods — All beaks used in the study were collected from squid caught in offsho.e and inshore Newfoundland waters. The squid were immature or in early maturing stages and covered a wide range of mantle lengths (see text). After extraction from the animals, the beaks were immersed for 2 d in a solution of pepsin to remove excess tissue and then stored in 70% alcohol to prevent dehydration.

Five beak dimensions (Fig. 1a, b) identical to those considered by Clarke (1962b), were measured (to the nearest 0.01 mm) for both the upper and lower beaks under a dissecting microscope using needlepoint Vernier calipers.

Analysis and results — The following abbreviations and notations were used:

X_j: ($j = 1$ for RL, 2 for HL, 3 for CL, 4 for WL, and

[1]Present address: Fisheries Research Branch, Department of Fisheries and Oceans, Ottawa, Ont. K1A 0E6.

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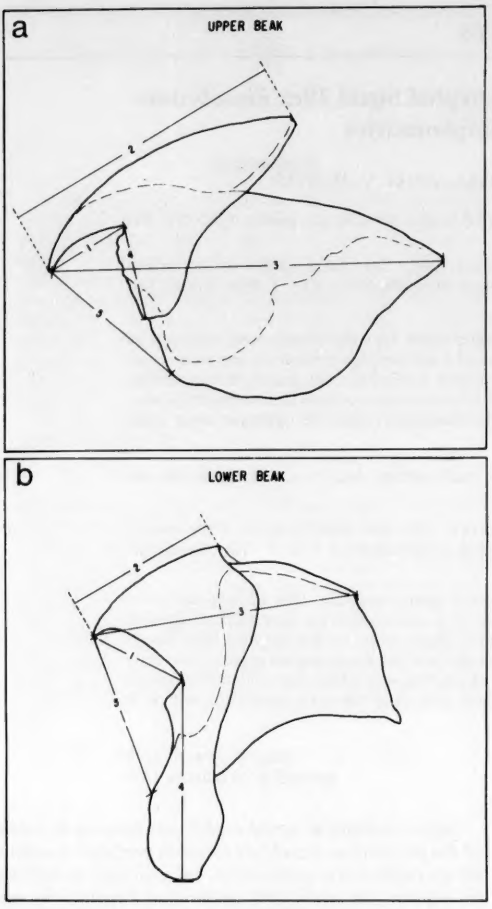

FIG. 1. Description of the measurements taken in this study of the upper beak (a) and the lower beak (b). RL = rostral length (1), HL = hood length (2), CL = crest length (3), WL = wing length (4), and RW = width of rostrum (5).

5 for RW) (see the legend of Fig. 1a, b for description of abbreviations)

r_{ij}: coefficient of correlation between X_i and X_j

There were 127 male and 190 female individuals with all the five measurements of the upper and the lower beaks available. A two-way classification multivariate ANOVA formed by sex and position (upper and lower) of the beak, with individuals "nested" within sexes tested whether the position of the beak is important (i.e. interaction is present) in comparing male and female beaks. Wilk's lambda criterion was employed for this nested-factorial layout (with the sources "sex"

TABLE 1. Means, standard deviations, and ranges of the upper beak measurements of the male and the female data (Units 0.1 mm).

Character	Male			Female		
	Mean	SD	Range	Mean	SD	Range
X_1	31.26	6.67	14–45	31.36	6.80	12–47
X_2	103.30	19.97	53–140	106.24	20.58	47–151
X_3	134.60	25.08	64–176	137.94	25.38	64–193
X_4	32.94	7.22	12–49	33.13	6.84	17–48
X_5	25.24	5.36	9–35	25.87	5.39	14–39

and "position" considered as fixed effects) to test the null hypothesis that sex × position interaction was absent. For an exposition of applications of Wilk's lambda in multivariate ANOVA see, e.g. Kshirsagar (1972). Estimate of the likelihood ratio test statistic $U_{(5,1,315)}$ was 0.3235, yielding $P \leq 0.01$. Significant interaction indicated that the difference between the vectors of upper and lower beak measurements in the males was not of the same magnitude as in the females. Therefore in the following statistical analysis measurements from upper beaks were considered separately from lower beak measurements in comparing the male and female beaks.

Male versus female, based on dimensions of the upper beak — There were 188 male and 266 female individuals with all five measurements on the upper beak available. Ranges of mantle length, viz. 9.0–22.6 cm (♂) and 8.5–23.9 cm (♀), overlapped. Mean mantle lengths (17.7 cm for ♂ and 18.1 cm for ♀) did not differ significantly ($P > 0.05$). Beak measurements (Table 1) were first analyzed for differences between sexes in the means of each individual dimension. When considered individually, no dimension indicated difference between sexes ($P > 0.05$). This was not surprising as ranges overlapped for all five dimensions (Table 1) and the dimensions were highly correlated, mutually. Within-sex r_{ij} ($i,j = 1,2, \ldots . 5; i \neq j$) ranged from 0.8815 to 0.9864. Each r_{ij} was significant ($P \leq 0.01$). "A series of univariate statistical analyses carried out separately for each of the variables is, in general, not adequate as it ignores the correlations among variables" (Kshirsagar 1972). Therefore it became necessary to do a multivariate ANOVA to test the significance of the difference between sexes in the vectors of means. An estimate of the likelihood ratio test statistic $U_{(5,1,452)}$ of 0.9394 indicated that males and females differed in their mean values ($P \leq 0.001$).

These preliminary observations indicated that a combined response, $\Sigma\, a_i x_i$ ($i = 1,2, \ldots . 5$), may make the identification of sexes possible where single measurements did not. As the two samples were identified on the basis of sources other than these five dimensions, discriminant analysis was employed to provide such a compound criterion (Kendall and Stuart 1975;

TABLE 2. Means, standard deviations, and ranges of the lower beak measurements of the male and the female data (Units 0.1 mm).

Character	Male			Female		
	Mean	SD	Rage	Mean	SD	Range
X_1	30.67	5.92	17–44	31.23	5.98	14–44
X_2	35.93	7.35	20–51	37.20	7.87	18–57
X_3	68.14	14.28	34.98	70.36	15.01	33–103
X_4	59.11	11.92	29–81	61.39	12.54	28–88
X_5	24.34	4.94	13–34	25.11	5.00	10–37

Rohwer 1972). The discriminant coefficients a_i were estimated by maximizing the ratio of the compound response to its standard error. Discriminant analysis has a close analogy to multiple regression as explained, e.g. in Kshirsagar (1972). Computations were done following the regression procedure by defining the difference between two samples in terms of arbitrarily chosen values of a "dummy" variate. The estimated discriminant function $-0.1245X_1 + 0.0379X_2 - 0.0035X_3 - 0.0453X_4 + 0.0948X_5$, was significant ($P \leq 0.001$). Estimates of mean discriminant scores and their standard errors were 0.4603 ± 0.0196 for the male and 0.5980 ± 0.0165 for the female. These statistics can be used effectively in identifying the sex of an individual from its upper beak measurements.

Male versus female, based on dimensions of the lower beak — Lower beak data yielded results which were similar to those obtained from the upper beak data. There were 217 male and 302 female individuals with all five measurements on the lower beak available. For the two sexes, ranges of mantle length overlapped (9.3–22.6 cm for ♂ and 8.3–23.4 for ♀) and means (17.6 cm for ♂ and 18.1 cm for ♀) did not differ significantly ($P > 0.05$). There were no differences between sexes in the means of individual lower beak dimensions (Table 2) except for X_4 ($P \leq 0.05$). Ranges of measurements overlapped (Table 2) and within-sex, r_{ij} values were high, ranging from 0.9016 to 0.9660. Each r_{ij} was significant ($P < 0.01$). Sexes differed in the vectors of means of five measurements ($U_{(5,1,517)} = 0.9757$, $P < 0.05$). The estimated discriminant function, $-0.1731X_1 + 0.0354X_2 - 0.0379X_3 + 0.0713X_4 + 0.1297X_5$, was significant ($P \leq 0.05$). Mean discriminant scores and their standard errors were 0.7490 ± 0.0269 for the male and 0.8753 ± 0.0228 for the female.

The algorithm and the computer program were written by R. K. Misra. This program may be obtained by contacting him.

Discussion — The results of these analyses demonstrate that discriminant function analysis of the dimensions of either the upper or lower beak can be used effectively in determining the sex of the individual *Illex illecebrosus* from which a particular beak came. However, the upper beak dimensions seem to discriminate between the sexes to a larger extent than the lower beak dimensions ($P \leq 0.001$ for upper beak vs. $P \leq 0.05$ for lower beak).

The identification of beaks into families can be done readily (Clarke 1962b). Because the only abundant species of the family Ommastrephidae in Newfoundland waters during the summer is *Illex illecebrosus* and a major predator, the pilot whale (*Globicephala melaena*), restricts its diet almost exclusively to *Illex illecebrosus* (Sergeant 1962), it should be easy to identify beaks tentatively to the species level. A comparison with beaks from complete animals and examination of the bodies of partly digested animals should also aid in beak identification. The pronounced hood evident on the upper beak (Fig. 1a) distinguishes it from the lower beak (Fig. 1b) at a glance.

Since predators especially pilot whales can consume hundreds of thousands of tons of *Illex illecebrosus* annually (Mercer 1975) it may be possible using this technique to explain areal and seasonal differences in sex ratios observed in squid populations in Newfoundland waters.

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Chromosome Complement of the Marine Worm *Neanthes arenaceodentata* (Polychaeta: Annelida)

GERALD G. PESCH AND CAROL E. PESCH

U.S. Environmental Protection Agency, Environmental Research Laboratory, Narragansett, RI 02882, USA

PESCH, G. G., AND C. E. PESCH. 1980. Chromosome complement of the marine worm *Neanthes arenaceodentata* (Polychaeta: Annelida). Can. J. Fish. Aquat. Sci. 37: 286–288.

The chromosome complement for the marine worm *Neanthes arenaceodentata* consists of nine pairs; one pair has a median centromere, seven pairs have submedian centromeres, and one pair is polymorphic with either a subterminal or terminal centromere. A technique for studying polychaete chromosomes is described. This species was selected as a model for studying cytogenetic effects of toxicants in marine environments because it has a suitable karyotype, is easily cultured, and as a benthic organism it is representative of an ecologically important group of organisms.

Key words: polychaete, chromosomes, karyotype

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Le complément chromosomique du ver marin *Neanthes arenaceodentata* est formé de neuf paires; une paire possède un centromère médian, sept paires des centromères submédians, et une paire est polymorphe avec centromère soit subterminal, soit terminal. Nous décrivons une méthode d'étude des chromosomes des polychètes. Cette espèce a été choisie comme modèle d'étude des effets cytogénétiques de toxiques en milieux marins, parce qu'elle possède un caryotype approprié, s'élève facilement et, comme organisme benthique, est représentative d'un groupe d'organismes écologiquement importants.

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ALTHOUGH the family Nereidae occurs worldwide and includes 439 species within 37 genera (Fauchald 1977) little information exists relative to cytogenetic properties. Bonnevie (1907) reported a diploid chromosome number of 20–30 for the species *Nereis limbata* and Just (1915) found a haploid chromosome number of 14 for *Platynereis megalops*. Nearly 50 yr later, Ometz (1963) determined a diploid chromosome number of 32 for *Nereis diversicolor*, 34 for *Perinereis cultrifera*, and Davis (1969) reported a diploid chromosome number of 18 for *Neanthes arenaceodentata*. However, none of the authors presented karyotypes.

This note contains details of the cytological technique employed for obtaining a karyotype and a description of the chromosome complement of *Neanthes arenaceodentata* from somatic cells arrested at metaphase.

Materials and methods — The test animals (*Neanthes arenaceodentata*, Moore) were from laboratory cultures originally obtained from Dr Donald Reish at California State University, Long Beach. *Neanthes arenaceodentata* construct mucous tubes in sediment or on firm surfaces such as algal mats or mussel beds. Females deposit eggs within the mucous tubes, then die. Males then incubate the eggs and the larvae develop within the tubes.

Larvae are removed from the tubes after 8–10 d with a siliconized Pasteur pipette and treated with 0.05% colchicine in a polyethylene beaker containing 100 mL of 30‰ filtered seawater for approximately 12 h at room temperature. The following procedure for slide preparation is adapted from Kligerman and Bloom (1977). Seawater is

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decanted and replaced with 100 mL of 0.075 mol/L solution of potassium chloride for 1 h, followed by larval fixation in three changes of ethanol–acetic acid (3:1) for 1 h each. Fixed larvae (25–30) are then placed on a clean slide and enough 50% acetic acid added to cover all of the larvae (several drops). After 4 min the acid is drained off and the larvae are smeared by pressing a second slide on top of the first and then drawing the two slides apart with one smooth continuous movement. This produces a monolayer of separated cells on each slide, which are air dried and stained for 5 min in 5% Giemsa made up in deionized water. These slides are rinsed thoroughly in deionized water, air dried, dipped in xylene, and mounted in coverbond. Photographs were taken with Kodak 35-mm high contrast copy film using a Zeiss Universal scope, 100× planapochromatic objective, 1.6× optovar, and 10× ocular.

Results and discussion — A diploid chromosome number of 18 was observed in cells arrested in metaphase in 25 different worms. This agreed with the observation of Davis (1969). A representative karyotype for *N. arenaceodentata* is shown in Fig. 1. The chromosomes range in size from 4 to 7 μm, and can be grouped into two size categories. One group consisting of pairs, No. 1–5, measure from approximately 6 to 7 μm and the other group, No. 6–9, measure from approximately 4 to 5 μm. The chromosomes are described according to the terminology of Levan et al. (1964). The centromere of the largest chromosome, No. 1, is located in the median region. Chromosomes No. 2–8 have centromeres located in the submedian region (Table 1). The shortest chromosome, No. 9, appears in two forms (Fig. 1). One form has a short second arm with centromere in the subterminal region; the second form appears to have a terminally located centromere. The shortest chromosome pair of individual worms consisted of either a combination of one of each type or both with terminal centromeres. We have never observed short pairs where both chromosomes contained subterminal centromeres. We do not know the significance of the differences observed in this chromosome pair.

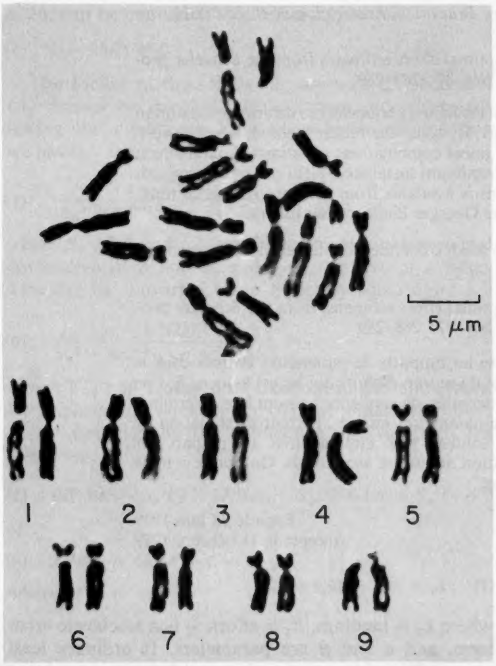

FIG. 1. Chromosomes of *Neanthes arenaceodentata* numbered from largest to smallest.

TABLE 1. Length and centromere position of *Neanthes arenaceodentata* chromosomes.

Chromosome number	Length (μm)	Arm ratio[a]	Centromere location[a]
1	6.7	1.56	median region
2	6.1	1.84	submedian region
3	6.0	2.20	submedian region
4	5.8	2.15	submedian region
5	5.7	2.89	submedian region
6	4.9	2.93	submedian region
7	4.6	2.72	submedian region
8	4.2	2.40	submedian region
9A	3.6		terminal point
9B	4.5	3.67	subterminal region

[a]According to Levan et al. (1964).

Neanthes arenaceodentata was chosen as a model to study cytogenetic effects of toxicants in marine environments because it has a suitable karyotype (18 large chromosomes) and is easily cultured, thus providing a continuous supply of experimental material. This species has many attributes which make it valuable to assess effects of pollutants. Because *N. arenaceodentata* is a benthic species, it is of particular interest because sediments are often reservoirs for pollutants and polychaetes are important food web species for commercially important fishes. Consequently, this species is widely used in marine toxicological studies (Reish et al 1976; Oshida et al. 1976; Rossi and Anderson 1976, 1977; Neff et al. 1978; Pesch 1979). We are presently developing a short term, cytogenetic assay technique, sister chromatid exchange responses (SCE), for *N. arenaceodentata*. Because an SCE assay may be conducted in vivo, it may be possible to relate cytogenetic observations to subsequent biological consequences inflicted by genetic toxicants at the individual and population levels.

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Confidence Intervals for Optimal Effort Estimates from the Schaefer Production Model

RICHARD L. MCGAW

Department of Economics, University of New Brunswick, Fredericton, N.B. E3B 5A3

MCGAW, R. L. 1980. Confidence intervals for optimal effort estimates from the Schaefer production model. Can. J. Fish. Aquat. Sci. 37: 288–289.

The Schaefer production model uses ratios of estimated parameters to determine maximum sustainable yield and the associated level of effort. By using the Fieller method, whereby confidence intervals can be determined for ratios of linear combinations of estimated parameters, the error component of the estimate of effort at maximum sustainable yield can be determined. The information needed to make the calculations is available from standard output of most regression programs. An example is given for the Georges Bank scallop fishery.

Key words: Fieller method, Schaefer production model, confidence interval, fisheries management

MCGAW, R. L. 1980. Confidence intervals for optimal effort estimates from the Schaefer production model. Can. J. Fish. Aquat. Sci. 37: 288–289.

Le modèle de production de Schaefer utilise les rapports de paramètres estimés dans la détermination du rendement maximal soutenu et du niveau d'effort qui lui est associé. Si l'on utilise la méthode de Fieller, selon laquelle les intervalles de confiance peuvent être déterminés pour des rapports de combinaisons linéaires de paramètres estimés, l'erreur d'estimation de l'effort correspondant au rendement maximal soutenu peut être mesurée. La plupart des programmes de régression fournissent l'information nécessaire aux calculs. On donne comme exemple la pêcherie de pétoncle du banc Georges.

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THE Schaefer production model is widely used in economic modeling of fisheries. The form of this production function is:

$$(1) \quad L_t = \alpha E_t - \beta E_t^2 + \epsilon_t$$

where L_t is landings, E_t is effort, ϵ_t is a stochastic error term, and α and β are parameters. If ordinary least squares is used to estimate this model, it is assumed that ϵ_t is a normally distributed random variable with mean

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equal to zero and a constant variance. If this assumption is correct, the estimates of α and β are unbiased, consistent, normally distributed, and have minimum variance. They are, however, statistical estimates of the true positive parameters and may overlap zero. While the analysis of this problem is well-known, much less is known about the distribution of ratios of these statistical estimates.

In the Schaefer model, estimates of maximum sustainable yield (MSY) and the level of effort as MSY (E^*) are obtained from ratios of parameter estimates:

$$\text{MSY} = \frac{a^2}{4b} \tag{2}$$

$$E^* = \frac{a}{2b} \tag{3}$$

where a and b are least-squares estimates of α and β, respectively. Since these results are important for policy implications from the model it would be useful to have confidence intervals for the values of MSY and E^* to determine whether they are significantly different from zero.

Assuming that all least-squares assumptions hold, the following method known as the Fieller method and adapted from Blomqvist (1973), determines confidence intervals for ratios of linear combinations of estimated parameters. The initial hypothesis, $E^* = a/2b$, can be converted to a linear hypothesis:

$$a - 2bE^* = 0 \tag{4}$$

The Fieller method finds all values of E^* such that (4) cannot be rejected. Using known procedures for testing the confidence limit of a linear combination we have:

$$\frac{a - E^*(2b)}{(S_a^2 + 4E^{*2}S_b^2 - 4E^*S_{ab})^{1/2}} > t_x \tag{5}$$

where S_a^2, S_b^2 and S_{ab} are the estimated variances and covariance of a and b, and t_x is the critical t value. This can be converted to an F test by squaring:

$$\frac{[a - E^*(2b)]^2}{S_a^2 + 4E^{*2}S_b^2 - 4E^*S_{ab}} > F_{2x} \tag{6}$$

where F_{2x} has 1 degree of freedom in the numerator. By imposing an equality on (6), we obtain all values of E^* bounded by the confidence interval from the following:

$$4E^{*2}(b^2 - S_b^2F) - 4E^*(ab - S_{ab}F) + (a^2 - S_a^2F) = 0 \tag{7}$$

or

$$4AE^{*2} - 4BE^* + C = 0 \tag{8}$$

where $A = b^2 - S_b^2F$

$$B = ab - S_{ab}F$$

$$C = a^2 - S_a^2F$$

This is a quadratic in E^* and the solution reduces to:

$$E^* = \frac{B \pm (B^2 - AC)}{2A} \tag{9}$$

Given the standard output of most regression programs, the confidence interval for E^* can be calculated. Because the estimate of MSY is obtained from a nonlinear combination of parameters, the Fieller method cannot be applied. Further details on the properties of the Fieller method are given by Blomqvist (1973).

For illustration, the Fieller method is applied to a Schaefer model estimated for the Georges Bank scallop fishery (McGaw 1978).

The estimate of the Schaefer model was:

$$L_t = \underset{(10.53)}{2.105}\, E_t - \underset{(2.53)}{0.0582}\, E_t^2 \tag{10}$$

with t-values given in brackets. The values necessary to calculate the confidence interval are:

$a = 2.105$
$b = 0.0582$
$S_a^2 = 0.03995$
$S_b^2 = 0.00053$
$S_{ab} = 0.00445$
$F_{1,28}^{.05} = 4.2$

The values of A, B, and C are:
$A = 0.001161$
$B = 0.1038$
$C = 4.2632$

From (3), E^* is equal to 18.1 thousand boat-days, and from (9), the 95% confidence interval for E^* is 11.8–77.7 thousand boat-days. Unlike most confidence intervals, those from the Fieller method are nonsymmetric about the point estimate.

The usefulness of the Fieller method is that it emphasizes the fact that the estimates of effort and MSY are statistical point estimates and may not be significantly different from zero. Thus when using a Schaefer model or any of its variants, care should be taken in the interpretation of the results for purposes of fisheries management.

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Techniques for Exploring and Presenting Data Applied to Lake Phosphorus Concentration[1]

K. H. RECKHOW

Department of Resource Development, Michigan State University, East Lansing, MI 48824, USA

RECKHOW, K. H. 1980. Techniques for exploring and presenting data applied to lake phosphorus concentration. Can. J. Fish. Aquat. Sci. 37: 290–294.

Water quality sampling and data analysis are undertaken to acquire and convey information. Therefore, when data are presented, the form of this presentation should be such that information transfer is high. For example, a graph or table of average values is often an inadequate summary of batches of data. As an alternative, a technique is presented (that was developed for exploratory data analysis purposes) that can be used to display several sets of data on a single graph, indicating median, spread, skew, size of data set, and statistical significance of the median. This technique is useful in the study of phosphorus concentration variability in lakes. Additions to, and modifications of, this procedure are easily made and will often enhance the analysis of a particular problem. Some suggestions are made for useful modifications of the plots in the study and display of phosphorus lake data and models.

Key words: limnology, exploratory data analysis, statistics, phosphorus, water quality, models, lakes

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L'échantillonnage et l'analyse des données touchant la qualité de l'eau ont pour objet l'acquisition et la dissémination de l'information. Lorsqu'il s'agit de présenter les données, la forme adoptée doit être de nature à assurer un transfert efficace de l'information. Par exemple, un graphique ou un tableau de valeurs moyennes résume souvent mal un groupe de données. L'auteur décrit une autre méthode (mise au point en vue d'une analyse préliminaire des données) pouvant afficher plusieurs séries de données en un seul graphique, indiquant la médiane, l'étendue, la symétrie, le volume de données et la signification statistique de la médiane. Cette méthode est utile dans l'étude de la variabilité de la concentration de phosphore dans les lacs. On peut facilement ajouter à cette méthode, ou la modifier, ce qui améliorera souvent l'analyse d'un problème particulier. L'auteur fait quelques suggestions touchant d'utiles modifications aux tracés dans l'étude et l'exposition de données et modèles du phosphore des lacs.

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DATA or statistics are presented to convey information. This information transfer process is most effective when a good balance is achieved between the amount of information conveyed and the succinctness of the presentation. Ideally, one would present the "sufficient statistics" as a summary of a set of data on a single variable, since, by definition, the sufficient statistics represent all of the information contained in the complete data set. For example, if the data are normally distributed, the sufficient statistics are the mean and the standard deviation of the data set. However, it is rare that actual data distributions can be exactly characterized by a theoretical density function (e.g. the normal distribution), so the researcher must usually select sample statistics that maximize information transfer.

In this paper, lake phosphorus concentration data in north temporate lakes are examined and displayed using various procedures. One technique, the "box plot" (McGill et al. 1978) is found to be particularly effective in conveying information about a set of data. Variations on the box plots are proposed that may improve the analysis of the problem under study.

Lake data analysis — Subsequent to the collection of data, the researcher faces the problem of summarizing the data set in a few numbers (or statistics) that hopefully result in minimal information loss in this translation from complete data set to sample statistics. If a single number is used to represent a set of data for a single variable, it is almost always a measure of the central tendency (e.g. the average value). Often, the (arithmetic) mean (represented by circles) or the geometric mean (mean of $\log_{10}$ transformed data, represented by triangles) is used to summarize data. For example, these two statistics are presented in Fig. 1a for phosphorus concentration measurements taken in six lakes (each lake is identified by its mean depth,

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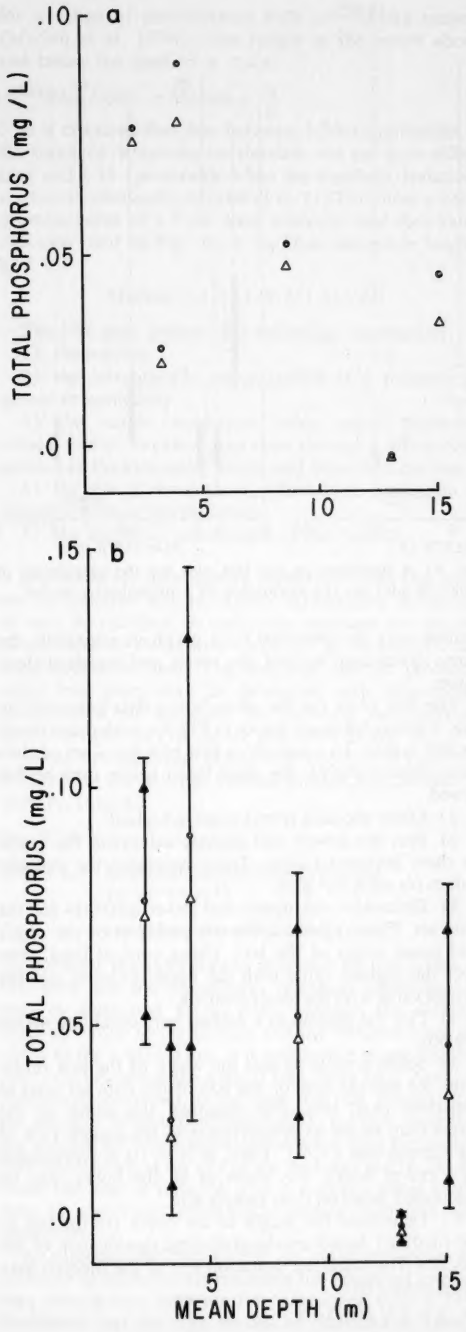

plotted on the x-axis). These points are estimates of the "location" of the data, but each alone contains no information on:

1) the variability of the data around this average. The data could be spread widely about the mean, or all data points could be equivalent and equal to the mean value.

2) the skew in the data. The data could be symmetrically distributed about the mean, or highly asymmetric.

3) the number of data points. As a rule, the "robustness" of a statistic is a function of the amount of data used in the estimation of that statistic. Thus, for a particular statistic, the robustness increases as the number of data points increases.

In addition, if the researcher chooses to use a measure of central tendency to represent a data set, a decision must still be made on the choice of the central tendency statistic. Specifically, for most real data sets, the mean, median, mode, and geometric mean will be represented by different numerical values, yet are candidates for this measure of location. The mean is most often selected; yet, if the formula for the calculation of the mean is examined:

$$(1)\quad \text{mean} = \frac{1}{n}\sum_{i=1}^{n} x_i,$$

it should be apparent that a single extreme value for x can significantly affect the mean. Thus, for highly skewed distributions, the mean may be overly influenced by an extreme data point, and it will not summarize the rest of the data well. For that reason, the geometric mean was also presented in Fig. 1a, as skewed data are often transformed logarithmically to reduce the skew. For that same reason, some statisticians have suggested that certain "robust" statistics be used when the shape of the distribution is either irregular or uncertain. A robust statistic is a statistic that serves its intended purpose fairly well under a variety of conditions. Thus, a robust statistic for the central tendency provides a good estimate of location for a set of data for a range of data distributions. An example of a robust statistic is the median, or middle value. The robustness of the median is apparent when one realizes that the median is unaffected by the particular values of the data points at the extremes of the distribution. The technique advocated herein for the display of data uses the median as the measure of central tendency.

Many researchers have realized that a single statistic for central tendency is an inadequate summary of a set of data on a single variable. Therefore, a second statistic, the standard deviation, is sometimes presented to characterize the variability in the data. Figure 1b is an example

FIG. 1. a) The mean and geometric mean for total phosphorus concentration; b) The mean and standard deviation for untransformed and $\log_{10}$ transformed data for phosphorus concentration in six lakes.

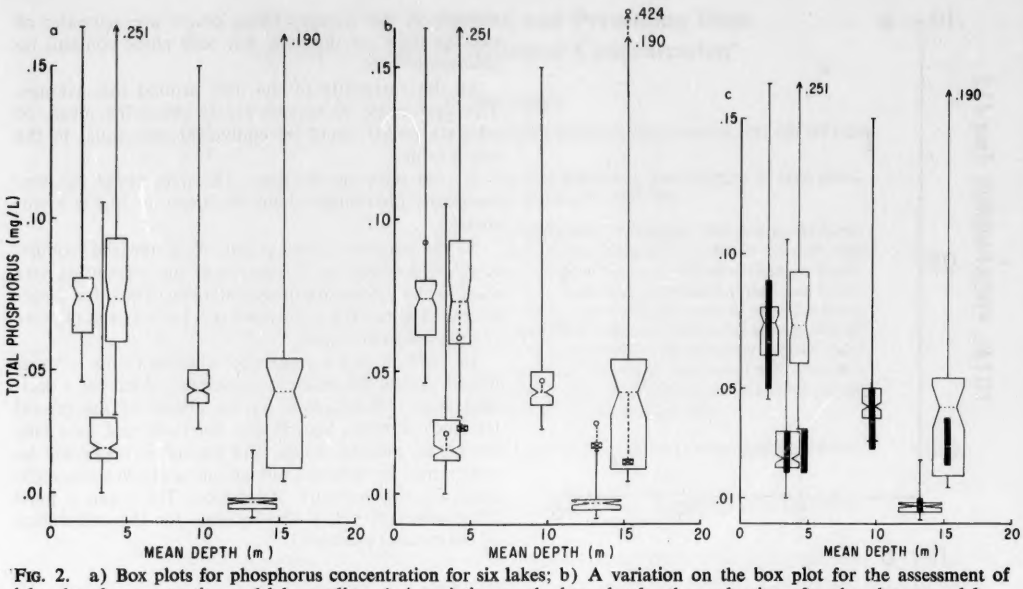

FIG. 2. a) Box plots for phosphorus concentration for six lakes; b) A variation on the box plot for the assessment of lake phosphorus retention and lake quality; c) A variation on the box plot for the evaluation of a phosphorus model.

of this approach, with the mean and the standard deviation (represented by bars), and the geometric mean with the standard deviation of the $\log_{10}$ transformed data (represented by solid triangles), used to represent the same phosphorus data presented in Fig. 1a. Of the three criticisms of Fig. 1a mentioned above, note that the first one is apparently addressed with the addition of either standard deviation, while the second and third criticisms are still valid for Fig. 1b. The first criticism concerning data variability is only apparently addressed because the standard deviation, like the mean, is not the most robust measure of variability, or spread. The standard deviation of the untransformed data will also be unduly affected by outlier data points, as the mean is. A robust measure of spread that should be considered in place of the standard deviation for many real data sets is the interquartile range, I (Mosteller and Tukey 1977). Both the median and the interquartile range are functions of order statistics; that is, they are based on an ordering of the data points from low value to high value. When the data are arranged like that, the median is the middle value (i.e. at the 50% level), and the interquartile range is the difference between the value at the 75% level and the value at the 25% level.

The box plot — This discussion forms a logical introduction to the technique recommended herein for the presentation of data. This technique, called the "box plot" (McGill et al. 1978), is constructed largely from the order statistics. It provides information on the median, spread or variability, skew, size of data set, and statistical significance of the median. All of this information may be conveyed on a graph in essentially the same space used to plot the mean and standard deviation.

The box plots for the phosphorus data presented in Fig. 1 for six lakes are drawn in Fig. 2a, with mean depth on the x-axis. To construct a box plot for a set of data on a single variable, the steps listed below may be followed.

1) Order the data from lowest to highest.

2) Plot the lowest and highest values on the graph as short horizontal lines. These represent the extreme values for each box plot.

3) Determine the upper and lower quartiles for the data set. These values define the positions of the upper and lower edges of the box. Using vertical lines, connect the highest value with the upper quartile and the lowest value with the lower quartile.

4) Plot the median as a dashed horizontal line within the box.

5) Select a scale so that the width of the box represents the sample size, or the size of the data set used to construct each box. For example, the width of the boxes may be set as proportional to the square root of the sample size ($\sqrt{N}$). Then, if $N = 10$ is represented by 1 cm of width, the width of all the boxes may be calculated based on their sample size.

6) Determine the height of the notch (in the box at the median) based on the statistical significance of the median. The standard deviation (s) of the median may be estimated by:

(2) $s = 1.25\, I/1.35\sqrt{N}$

for a range of distributions with normal-like centers (McGill et al. 1978). The height of the notch above and below the median is $\pm Cs$:

(3) Notch Limits = Median $\pm$ Cs

C is a constant that lies between 1.96 (appropriate if the standard deviations for the data sets are quite different) and 1.39 (preferable when the standard deviations are nearly identical). McGill et al. (1978) chose a compromise value of 1.7 for their example, and that value was also used in Fig. 2a, b, c. Thus the notch heights are:

$$\text{Median} \pm 1.7\ (1.25\ I/1.35\sqrt{N})$$

The box plots present the following information:

1) the median
2) the interquartile range, which is a measure of spread or variability
3) the range (maximum value minus minimum value), and an impression of skew through a visual comparison of the symmetry above and below the median
4) the size of the data set, which is an indication of the robustness of the statistics
5) the statistical significance of the median.

Box plots for lake quality analyses — The box plots are an effective way to express information about sets of data. In addition, though, they represent an example of the value of thoughtful creativity in the analysis and presentation of data. The study of specific problems using box plots may be enhanced with appropriate variations of the box plots. For example, two additions to the box plot in Fig. 2a are presented in Fig. 2b.

In the first addition, circles are attached to each box to represent the average influent phosphorus concentration, P_{in} (mg/L).

(4) $P_{in} = L/q_s$

where L = annual areal phosphorus loading ($g \cdot m^{-2} \cdot yr^{-1}$)

q_s = annual areal water loading (m/yr)

To consider the information provided by this addition, first note that the phosphorus retention coefficient, R, may be estimated as the ratio between the distance from the circle to the median and the distance from the circle to the x-axis. Thus, it is interesting to see whether the circle lies above the highest lake value (e.g. Are concentrations observed in the lake that are greater than the average influent concentration?). Further, note that the average influent concentration is *less* than the median lake concentration for one of the six lakes in Fig. 2b. An event like that is unusual, and it suggests either faulty data or significant phosphorus release from the lake sediments.

As a second addition to Fig. 2a, small rectangles are attached to each box representing the predicted average lake phosphorus concentration associated with a 75% likelihood that the lake should be classified as anoxic. This is calculated from the oxic–anoxic discriminant analysis (Reckhow 1978) that was used to derive a relationship to estimate the probability that a lake should be classified as oxic or anoxic. This relationship is:

(5) $$Pr_{oxic} = \frac{1}{1 + 10^{5}\, z^{-2.49}\, L^{2.00}\, q_s^{-1.78}}$$

where Pr_{oxic} = probability that a lake should be classified as oxic ($= 1 - Pr_{anoxic}$)

z = lake mean depth (m)

If the anoxic probability is set at 0.75 (generally indicative of severe oxygen depletion and possible anoxia), and q_s and z are constant, then the phosphorus loading associated with an anoxic probability of 0.75 is calculated from:

(6) $L = \sqrt{3 \times 10^{-5}\, z^{2.49}\, q_s^{1.78}}$

The predicted lake phosphorus concentration resulting from this loading (associated with $Pr_{anoxic} = 0.75$) is then calculated from (Reckhow 1979):

(7) $$P = \frac{L}{11.6 + 1.2q_s}$$

where P = lake phosphorus concentration (mg/L).

This value was determined for only three of the six lakes presented in Fig. 2b because Equation 5 is "valid" for lakes only with q_s between 1 and 50. The resultant rectangle on Fig. 2b provides an indication of the current quality of each lake. This quality may be assessed by answering the following questions. Is the rectangle above or below the median? (e.g. Is the anoxic probability for the present condition of each lake less than 0.75 or greater than 0.75?) Is the rectangle inside or outside the box? (e.g. How "far" is each lake from $Pr_{anoxic} = 0.75$?) A graph like Fig. 2b can provide answers to these questions.

Another application for the box plot is as a graphical technique for the examination of the fit from a proposed model for the variable on the ordinate. For example, the model in Equation 7 may be used to predict the phosphorus concentration for the six lakes. These predictions can be plotted on the basic box plot in Fig. 2c, along with error bars representing the standard error of the model ($s = 0.128$ for the log-transformed model). For each lake, the space between the bars represents the predicted concentration, or the prediction from the model for the median concentration. The difference between this space and the median (at the center of the notch for each box) is the model residual.

The residual is generally thought to be the best measure of fit of a model to a particular set of data. However, the box plots and error bars indicate an alternative measure of fit, which is the correspondence between the box and the error bars. For instance, the model provides an excellent fit to the data for Lakes 2 and 5 (the lakes are numbered from left to right) because the medians and predictions correspond closely,

as do the error bars and boxes. In addition, the fit is extremely poor for Lake 3, which is not surprising given the information conveyed on this lake in Fig. 2b. The fit for Lake 1 is fairly good since the error bars encompass the median and the entire box. Thus the phosphorus concentration prediction presented for this lake, along with the prediction interval, correspond with the majority of phosphorus measurements obtained for the lake. Therefore, while the fit for Lake 1 appears only fair on the basis of the residual, it looks good when the region of observation and prediction are compared.

On the other hand, the fit for Lake 4 has a mixed interpretation. While the predicted phosphorus concentration is close, in magnitude, to the median observed concentration, it is still outside the box, suggesting a poorer fit (than is apparent from examination of the residuals alone). Note that the error bars cover both the median and points below the minimum observed value. Likewise, the fit for Lake 6 is mixed in its success. The predicted phosphorus concentration is far from the median observed concentration, and as a result, the error bars do not even cover the median observed value. Yet, the predicted concentration and error bars are entirely contained within the box. This means that the prediction and prediction interval correspond with the middle 50% (actually the second 25%) of the observed values. Thus the prediction and prediction interval are representative of observed concentrations in Lake 6.

Despite the fact that the effectiveness of the model is being judged according to the correspondence between the error bars and the box, the two characteristics do not represent the same quantity. The prediction interval, which is defined by the error bars, represents the uncertainty in the prediction of the statistic representing location (e.g. the average or the median). The box, or the interquartile range, represents a range of concentrations observed in a lake. Thus, the error bars provide an estimate of the uncertainty in a single statistic (like the notch does for the median), while the box represents the variability in a set of data. However, while one should not regard the error bars and boxes as representations of the same quantity, it is instructive to compare these characteristics. Specifically, to the extent that a prediction of lake phosphorus concentration (and its associated prediction interval) is intended to characterize the phosphorus concentration in a lake, the correspondence between that prediction and the set of observed values can only aid interpretation of the model fit. Since residuals analysis involves the comparison of a *point* prediction with a point observation, this procedure should improve the analysis as it is based on a comparison of a prediction *region* with a region of observation.

Concluding comments — The basic graphical procedure presented herein, the box plot, may be used to both examine data and to display characteristics of a set of data. Variations on the box plots are presented in the last section and a number of specific uses of the box plots are mentioned. As suggested above, it is important that statistics and graphical displays be carefully chosen for the analysis and presentation of information. The techniques described herein should be effective transmitters of information and hopefully will lead to additional developments in this area.

Acknowledgments — The author wishes to acknowledge the assistance of the following graduate students in Resource Development: Michael N. Beaulac, Douglas Hyde, Russell Kruska, and V. David Lee. This research was supported by the National Oceanic and Atmospheric Administration, under Grant No. 03-78-B01-109.

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LETTERS/LETTRES

Comment on Estimated Escapement of Atlantic Salmon (*Salmo salar*) for Maximum Smolt Production in Rivers of Different Productivity

COMMENT

Symons (1979) dismissed my compensatory mortality model for juvenile salmon (Gee et al. 1978) on the grounds that I had confused movement with mortality and that the egg deposition densities the model implied exceeded the maximum he considered for whole rivers. Unfortunately, Symons has misinterpreted some of my data and miscalculated survival rates between the egg and fry stages.

Though some loss from the study sites may have been due to emigration the high densities observed throughout the study area suggest that migrants may not be able to contribute to production elsewhere. More recent studies on the main river as far as 30 km below the quoted study area confirm the presence of fry and parr in relatively high densities throughout the system. The majority of the study sites included pools as well as riffles so that the densities quoted are averages for the river as a whole and include movement from the spawning fords. Also, a recent study (Mawle 1979) involved trapping fry and parr moving in and out of a 580 m reach showed that the majority of the loss within the reach was probably attributable to in-site mortality.

Symons states that an optimum fry density for the Wye of 75/100 m² requires a minimum egg production of 375/100 m² for an assumed maximum egg-to-fry survival rate of 20% and more than 600/100 m² for suboptimal densities assuming an egg-to-fry survival rate of 13%. However, his rates apply to fry in August whereas the densities I propose apply to June. Recalculation shows the optimal egg deposition density to be 285/100 m² and for a suboptimal fry density of, say, 40/100 m² the required egg density is 246/100 m² (13% survival) or 159/100 m² (20% survival). These compare favorably with the average egg deposition density of 220/100 m² recommended by Symons for rivers producing 2+ smolts.

The compensatory model implies that total smolt output is reduced at high spawning intensity and can be shown to regulate population abundance in agreement with Elson's (1975) hypothesis. Clearly, the implications to management of salmon stocks are sufficiently important to warrant further investigation. — A. S. GEE, *University of Wales Institute of Science and Technology Field Centre, Newbridge-on-Wye, Powys, Wales.*

ELSON, P. F. 1975. Atlantic salmon rivers, smolt production and optimal spawning: an overview of natural production. Int. Atl. Salmon Found. Spec. Publ. Ser. 6: 96–119.

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REPLY

Gee is correct when he states that in calculating egg densities from fry densities for the River Wye, I mistakenly (Symons 1979) applied a survival to August rather than to June for the fry densities given in his paper (Gee et al. 1978). Using survival rates to June does bring estimated egg depositions in the Wye in line with those observed elsewhere, as Gee notes.

My suggestion of emigration in the Wye as an alternative to mortality for explaining disappearance of fish is valid in the absence of decisive information. If the new data cited by Gee satisfactorily eliminate the emigration or movement hypothesis, then his original conclusion is correct. Until then the question remains open.

The question "is there an optimum spawning or egg-deposition density which when exceeded results in decreased smolt production?" may be unanswerable today because no unexploited stocks with high density spawning remain. However, something more than simple compensatory mortality is required to explain optimum spawning; my model in fact already includes compensatory mortality as indicated by Elson's work in the Pollett River some 30 years ago. What is required is a mortality dependent on some initial density (say June fry density as suggested by Gee et al.) *which continues with little adjustment through intervening densities until populations are well below the potential carrying capacity of the river.* Data of Gee et al., if indeed reflecting mortality rather than movement, indicate this actually occurs; this is the potential importance of their work. Elson (1975) postulated how such an apparently unadaptive phonomenon might occur, but the supportive data he presented were, as he himself admitted, inconclusive.

My alternative hypothesis is that there is no optimum spawning density in the sense above (i.e. excluding the concept of "wastage"), but that smolt production will be neither decreased nor increased to any detectable degree by spawning densities in excess of my recommended minimal numbers (which may then be considered "optimum" if the concept of wastage is included).

Distinction between these hypotheses, as both Gee and I have noted, has important implications for management of salmon stocks. Further investigation is certainly warranted. — PHILIP E. K. SYMONS, *International Commission for the Conservation of Atlantic Tunas, Madrid, Spain.*

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Comment on Optimal Survey Design

COMMENT

I am concerned that the paper by Cuff and Coleman (1979) is likely to have an effect opposite to the authors' stated purpose of encouraging optimal survey design. Their results appear to be valid for the field study example given, but they also suggest that these results are valid in general, and that stratified sampling and replication of samples at stations are generally not worthwhile. Qualifying statements, which limit these generalizations to narrowly defined objectives of "survey sampling" studies, are made in the body of the paper but are not mentioned in the abstract. In any case, I question the suitability of the example as a basis for the generalizations.

The stratified design used in this study was (as the authors show) ineffective in improving precision of estimates of mean densities — for this study area, these variables, the strata as defined, and so on. It is known that stratified sampling *must* be more efficient than simple random sampling (srs) when strata are homogeneous within and heterogeneous among; i.e. when the mean square within strata is less than the mean square among strata. One must therefore conclude that in this case either the strata were poorly chosen or there was little large-scale spatial pattern in the levels of these variables, or both. Cuff and Coleman show that their initial stratification was inferior to strata redefined by faunal assemblages from a cluster analysis. This would seem to be an excellent approach, except that the clusters are presumably defined by all or most of the 500+ species distributions whereas the variables used to estimate "mean densities for the universe" are aggregates of these species into three higher taxa. Even if these redefined strata *are* close to optimal, it is likely that any distinct spatial patterns that were jointly defined by distributions of all the species would be greatly blurred (a reduced contrast between "homogeneity within strata" and "heterogeneity among strata") when described by a single aggregate variable such as "mollusks," resulting in a reduced gain in precision from stratification.

Since these "strata" appear to be heterogeneous within, one should perhaps treat the design as an area cluster sample where the "strata" are the areas. Each station then represents a cluster of potential grab samples from which three were selected. (The number selected must be a very small fraction of the potential number because the finite population correction has been omitted from the equation in Appendix 2.) Such a "by areas" design is usually not chosen for the sake of precision (as optimally defined strata in a stratified design would be) but rather for the sake of convenience or because the areas are of particular interest in some way (e.g. for the kinds of reasons given by the authors on p. 353 for choosing their original design). Since these objectives were apparently satisfied, and the authors reemphasize the desirability of "A stratified design ... in many instances" for these same reasons (p. 357), I find it odd that they then conclude that "The objectives assumed by survey statistics, and hence by this study, relate most closely to McIntyre's (1971) objective of making valid statements about the density ... of the fauna" — as opposed to McIntyre's other stated objective of assessing regional faunal density differences *within* the universe sampled.

Cuff and Coleman argue that "the most precise universe estimates can be obtained by taking only one grab sample at each station" (p. 357). This statement is subject to misinterpretation and I fear that it will be widely quoted, out of context. Presumably they mean "given some fixed total number of grab samples." Even if one considers only the precision of universe estimates, I again question the validity of generalizing from these results. An area cluster sampling design with more than one sample per cluster is less efficient than a srs design of the same size (with one sample per cluster but the same total number of samples) whenever the intracluster correlation is positive (Cochran 1963), i.e. when there is little small-scale (among-grabs within-stations) heterogeneity relative to among-stations within-areas heterogeneity. One would not obtain the same result using a stratified design where strata were defined so as to produce homogeneous among-stations within-stratum distributions on the criterion variable(s), with substantial small-scale patchiness at the scale of the area sampled by the grab. I do agree that overdefinition of stations by excessive replication is common, and should be avoided given the high cost of laboratory analysis. Precision increases in proportion to the square root of the number of replicates, which produces a very definite law of diminishing returns. Some replication at stations is of course necessary if for any reason one might wish to test for significance of density differences among stations or sets of stations using a posteriori tests, or if one wishes to assess small-scale patchiness distributions of species.

I doubt that there *are* general answers to the questions "Should I stratify?" or "How many replicates?", which is why some preliminary sampling is always advisable. I fear that inexperienced benthic ecologists who skim Cuff and Coleman's paper may use it to justify shortcuts, such as skipping preliminary sampling, not bothering to think about heterogeneity in the universe being sampled, and eliminating replicate samples. In many cases this would be a serious mistake, one which would be discovered only when it was too late.

I wish to thank J. C. Baskerville and R. E. Foreman for discussions and comments on this subject. However, the opinions expressed are my own. — ROGER H. GREEN, *Department of Zoology, University of Western Ontario, London, Ont. N6A 5B7.*

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Reply

I thank Dr Green for his comments. It would indeed be a mistake to regard our lessons as of proven general relevance, as we tried to point out in our paper (p. 352 of Cuff and Coleman 1979). Time and further random surveys will show whether generalizations are possible but, for now, it might clarify the way in which we see our results being useful to a wider audience if I retrace a few of the steps that led to our paper. An a posteriori look at the efficiency of various random survey designs for sampling Western Port led to two interesting results: that no random design was likely to produce substantially more precise universe estimates than any other and that, for fixed overall number of samples, the greatest amount of precision could have been obtained by allocating only a single grab sample per station. The second result suggested an optimal number of grab samples per station of only one, contrary to the common practice in benthic surveys of multiple grab samples per station.

Upon examination it became clear that the survey objective used by Saila et al. (1976) and assumed by our study (precise universe estimates) was at odds with that implied in the familiar cumulative- and recruitment-curve methods, with their emphasis on the definition of the station. This led us at first to wonder if, when using the precision criterion, it would be generally optimal to use one sample per station. (It is interesting that using cumulative- and recruitment-curve methods the Western Port data suggest at least three grab samples per station.) Having decided that it will be generally optimal to allocate only one sample per station for those surveys whose objective is precise universe estimates (see below), we began to think more carefully about the role of benthic surveys in environmental studies. The three objectives given by McIntyre (1971) are: to make valid statements about the number of species present, the distribution of the fauna, and the density (number of individuals and/or biomass per unit area) of the fauna. The objective of "assessing regional faunal density differences *within* the universe sampled" is subsumed within the last of McIntyre's objectives. The relative importance of each of these objectives in environmental studies is unclear and there is also doubt as to the spatial scale (local, regional, universe) of most interest and to the types of variables that are of greatest practical significance. We thought that our second lesson, in particular, could provide a useful basis for such a discussion.

Finally, we believed that the use of the cumulative and recruitment curves had in the past often led to the overdefinition of stations, with an unjustifiable dismissal of random designs as impractical. Once interest is generated and a number of random surveys are published I believe that general guidelines to effective benthic sampling designs will be found. But it will be some time before one may find in the literature the results of a number of random surveys and we hoped to shorten this search time by publishing our lessons as tentative suggestions that can be used and tested for general relevance in future surveys.

While in general I would encourage each benthic ecologist to undertake preliminary sampling to estimate the optimal number of samples per station, there is one case where untested acceptance of our second lessons seems warranted. In a number of applied surveys the benthic ecologist will not have the manpower/time to do adequate preliminary sampling. (This sentence should not be seen as encouraging more of such surveys.) For such surveys with objectives of obtaining precise universe estimates, the cumulative- and recruitment-curve methods will be inappropriate for defining the optimal number of samples per station. Then I would suggest, for lack of better information, that it is efficient to use one sample per station. (Since it is easy and inexpensive to collect extra samples at each station, it might be useful to do so but to process them only if a particular need makes it cost effective.)

Dr Green points out (his fourth paragraph) that if one could find a situation where the among-station variance (V_{1a} in Cuff and Coleman 1979) was less than the within-station variance (V_{2a}) then the predicted optimal number of samples per station would be greater than one. This is strictly only true if one does not consider cost information in the calculations. Nevertheless, the point is academic until a sufficient number of case studies have been generated. Available data (Table 2 of Saila et al. 1976) suggest that, in some cases, V_{2a} will be found to be as large as $2V_{1a}$. My guess would be that in practice V_{2a} will not commonly be sufficiently larger than V_{1a} so as to override the cost disadvantage against multiple samples per station.

I am obliged to co-author N. Coleman, and to R. R. Parker and S. Rainer for useful discussions. — Wilf Cuff, *CSIRO, Division of Computing Research, P.O. Box 1800, Canberra, A.C.T. 2601, Australia.*

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BOOK REVIEWS/COMPTES RENDUS

Book review editor:
David P. Scott
Department of Fisheries and the Environment
Fisheries and Marine Service
Freshwater Institute
501 University Crescent
Winnipeg, Man. R3T 2N6

***Tilapia.* A Guide to Their Biology and Culture in Africa.** *John D. Balarin (assisted by John P. Hatton). University of Stirling, Unit of Aquatic Pathobiology, Stirling, Scotland. 1979. xxiii + 174 p. £8.50.*

In this era of space age technology and rapid communication systems, it is important that subject reviews be prepared periodically to help scientists keep abreast of the massive proliferation of fisheries literature. Such reviews are important not only to catalogue appropriate references, but also to analyze existing information and identify gaps in knowledge which should be given priority in future research. The no-frills review of the biology and culture of tilapia species by John Balarin fits well into this mold.

Members of the tilapia group have in the past been important candidate species for fish culture because they were "relatively simple to cultivate, resistant to poor water quality and diseases, and able to efficiently convert many organic animal and agricultural materials into high quality protein." They were ultimately distributed widely from their indigenous regions in Africa to Asia and the Americas to further the cause of protein production. Despite disappointing yields resulting from uncontrollable reproduction, recent breakthroughs in overcoming this problem have resulted in tilapia again being viewed as a major culture species in developing countries. The approaches taken and depth of research in tilapia culture over the years could also serve as a model in most countries for developing an information base on new candidate species for aquaculture.

After introductory notes on world distribution, biology of principal tilapia species (which could have been included in later sections because of repetition), and usefulness of tilapia as a culture species, the meat of this book is found in the chapters describing ecological requirements, biological characteristics and culture systems for tilapia. Most of the literature reviewed is post Chimits' 1955 and 1957 bibliographies. Although the title implies an emphasis on application of information to Africa, important works from Israel, several Asian countries and the USA are reviewed. This is necessary because of the advances made in those countries on hybridization and monosex culture to counter the problem of early and excessive reproduction.

Information is collated for all tilapia species, with *Sarotherodon mossambicus* being the most widely used in research studies. While the authors have tried to separate knowledge on individual species whenever possible, the value of this book lies in the generalities on biology and culture of the genera. Reference to this text should make it possible to assess the potential suitability of a rearing site for tilapia culture, and subsequently plan culture strategy using the most appropriate techniques. Perhaps one shortcoming is that references related to technical details of facility construction and maintenance have not been reviewed although there are several good publications available.

On the subject of diseases, most emphasis is given to the many parasites which have been found during disease outbreaks in cultured tilapia, with *Ichthyophthirius*, *Costia*, and multicellular parasites being the most common. *Aeromonas hydrophila* is the only bacterial disease described as being isolated in cultured tilapia, although bacterial infections were suspected of being the cause of other epizootics. With the exception of lymphocystis, no viral diseases were described. Among fungal infections, those caused by *Saprolegnia* and *Branchiomyces* were reported. Since tilapia may be cultured in brackish water, blooms of algae producing toxins such as the "red tide" or toxins produced by blue-green algae are also of concern.

The authors sensibly do not get involved in the complex issue of taxonomy of tilapia. There is instead a short introductory note on this subject in which they choose to adopt Trewavas' (1973) proposal of classifying mouth-brooding tilapia in the new genus, *Sarotherodon*.

Apart from the text and list of 270 references, valuable supplemental information on tilapia is contained in the 17 figures (some are unfortunately difficult to interpret), 14 tables (several of which are multipage lists), and 14 appendices. This book will be as valuable to the scientist or fish farmer for its compilation and analysis of information as it will be to the student as a reference book. It should be a useful addition to the library of any agency, institution, or college having an interest in culture and management of tilapia populations.

CHIMITS, P. 1955. Tilapia and its culture: a preliminary bibliography. FAO Fish. Bull. 8: 1–33.
1957. The Tilapia and their culture, a second review and bibliography. FAO Fish. Bull. 10: 1–24.
TREWAVAS, E. 1973. On the cichlid fish of the genus *Pelmatochromis*, on the relationship between *Pelmatochromis* and Tilapia and the recognition of *Sarotherodon* as a distinct genus. Bull. Br. Mus. Nat. Hist. Zool. 25: 1–26.

T. G. CAREY
Department of Fisheries and Oceans
Fisheries Research Branch
Ottawa, Ont. K1A 0E6

S. F. SNIESZKO
U.S. Department of the Interior
National Fish Health Research Laboratory, Leetown
Kearneysville, WV 25430, USA

(R266)

Phytoplankton Manual. Monographs on oceanic methodology 6. *Edited by A. Sournia. United Nations Educational, Scientific and Cultural Organization (UNESCO), Paris, France. 1978. xvi + 337 p.*

This book arose from a working group of the Scientific Committee on Oceanic Research appointed to review current nonchemical methods for the quantitative study of phytoplankton, select the most satisfactory, and recommend

detailed procedures. Since the recommendation of detailed procedures appeared premature, a description and evaluation of existing methods was settled upon. This book is the result. Despite the broad title, it is directed toward the sampling, identification, and counting of marine phytoplankton. For the most part, the book fulfills its purpose of offering detailed descriptions of alternate procedures together with an evaluation of advantages and disadvantages. In many instances, the procedures are so detailed that anyone with little or no experience can follow them. But space limitations alone would make this impossible for all procedures and this has led to an uneven treatment and variable format. Some aspects, generally the simpler ones, are treated in great detail with listings of equipment needed and stepwise procedures. Others are given only general discussion with references to more detailed procedures.

The book treats in sequence the sampling, preserving, concentrating, identifying, and counting of phytoplankton, and interpretation of the results. Within each of these steps, the text is further subdivided into alternate approaches. As part of the introduction, the editor provides a useful guide to the various divisions and subdivisions. In some instances, this subdivision seems to have been taken too far, resulting in several 1- or 2-page notes which contribute little. In spite of this seemingly logical arrangement, there is some unnecessary repetition, as some contributors to the enumeration section repeat what has already been treated under sampling and concentration.

An introductory chapter on "Why Study Phytoplankton" seems extraneous; anyone taking up this book will already have his own reasons. However, congratulations are in order for including a discussion of sampling design, a topic which tends to be omitted from most compilations of methods. A discussion stressing the importance of a statistical basis for sampling design is nicely balanced against one on the practical limitations which must be met in planning a sampling program. In treating the collection and preservation of samples, the concentration and enumeration of the phytoplankton, and the making of permanent records, the emphasis is on the alternate approaches available. None is best in all situations but recommendations for making a choice in particular situations are given. The book concludes with a general discussion of the interpretation of results.

Throughout are brief discussions of statistics pertinant to each stage of the sampling program. Unfortunately, I doubt if these will be helpful to anyone who is not already familiar with statistics, the audience that should be reached. It might have been better to use the limited space which could be devoted to statistical considerations to give some examples of the greater insight which can be gained through the use of statistics, accompanied by references to more detailed procedures.

This book seems an accurate reflection of the present situation in the quantitative sampling of phytoplankton. No one approach can be recommended to the exclusion of others at any stage from sampling design to interpretation. The choices depend on the objectives of the study, the resources available, and the populations encountered. This book gives a variety of alternatives at each stage, detailing the simplest ones and giving general discussions, with references, for the more complex. Although the book, with the exception of a supplementary chapter, is restricted to the marine environment, much is equally applicable to fresh waters. While anyone with much experience in enumerating phytoplankton will probably not find much new here, it should provide a useful guide to newcomers or those with limited experience.

F. P. HEALEY

Department of Fisheries and Oceans
Freshwater Institute, Winnipeg, Man. R3T 2N6 (R278)

Reservoirs. *Brian Henderson-Sellers. MacMillan Press Ltd., London and Basingstoke, U.K. 1979. x + 128 p. £3.95.*

This book represents an admirable attempt to acquaint the uninitiated with the numerous disciplines that are united in reservoir planning, construction, and operation. Topics range from the history of water supply through dam design, economics, freshwater ecology, numerical modeling to flood routing among others. Of course, the expert in any one of these disciplines may be put off by errors or especially omissions which must occur in a book of this size which treats so many diverse topics. The author has obviously striven to keep the book a manageable size and on a necessarily superficial level so as not to discourage the layman. In these efforts he has been successful.

Discussions and examples in the text draw heavily on experience with British drinking water supply reservoirs. It is because of this experience that the author can offer the statement in the preface that "... the terms 'lake' and reservoir' may be regarded as interchangeable for our present purposes, and will be used as such." Anyone familiar with the Canadian scene must have difficulty accepting this equivalence. At best, reservoirs are artificial lakes manipulated to serve man rather than responding solely to nature's rhythms and whims. This is a fundamental difference which should never be forgotten. If reservoirs and lakes were truly interchangeable, then one might question the need for this book, given the several texts on limnology which have been appearing.

Although the author mentions the many purposes for which reservoirs may be created and does occasionally mention an example outside of Great Britain, the emphasis on domestic water supply does distort the treatment of several topics. A resource biologist will likely take offense when he reads the grudging admission (p. 88), "Some organisms are of course desirable ...." There is an implication throughout the sections on freshwater ecology and water quality that the ideal water is akin to distilled water. Desirable as this may arguably be in drinking water, it does give short shrift to the fisheries resources of reservoirs which can be immensely valuable in many tropical systems and which enhance the multi-use concept of modern reservoir management. The book also fails to recognize that reservoir formation and operation themselves can create water quality problems and resource deterioration.

In view of the diverse problems reservoirs pose as well as solve and their increasing ubiquity, the general public cannot avoid the impact of reservoirs on its life. We will all need a general understanding of reservoirs. This book will serve as a suitable introduction to domestic water supply reservoirs, but the layman would benefit from a similar level of treatment to the worldwide experience with reservoirs of all types.

R. E. HECKY

Department of Fisheries and Oceans
Freshwater Institute
Winnipeg, Man. R3T 2N6 (R283)

Books received/Livres reçus[1]
September/septembre 1979

***Biological Data in Water Pollution Assessment: Quantitative and Statistical Analyses.** *Edited by K. L. Dickson, John Cairns Jr., and R. J. Livingston. American Society for Testing and Materials (ASTM), Philadelphia, PA. 1978. vii + 184 p. $17.50.* (Proceedings of a symposium sponsored by ASTM Committee D-19 on water, at Minneapolis, Minn., June 20–21, 1977.)

Fishing Boats and their Equipment. *Dag Pike. Fishing News Books Ltd., Farnham, Surrey, England. 1979. x + 182 p. £6.25.* (Another in the series of small, practical books published by Fishing News Books Ltd. on various aspects of commercial fishing.)

Energy and Environmental Stress in Aquatic Systems. *Edited by James H. Thorp and J. Whitfield Gibbons. Technical Information Center, U.S. Dept. of Energy. (Available as CONF-771114 from National Technical Information Service, U.S. Dept. of Commerce, Springfield, VA 22161). 1978. xv + 854 p. $15.00.* (Selected papers from a symposium held at Augusta, Georgia, November 2–4, 1977.)

****Chondrus crispus* and carrageenan, a bibliography (1972–1978).** *B. E. Mackey and A. R. Taylor. Proceedings of the Nova Scotian Institute of Science, Vol. 29, Part 3. Nova Scotian Institute of Science, Halifax, N.S. 1979. p. 237–312. $15.00.*

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[1]Entries in this list *not* marked by an asterisk are available for review by interested readers for a 3-month period from the time of publication of the list. For information, please write to the Book Review Editor.

[1]Les ouvrages inclus dans cette liste et *non* accompagnés d'un astérisque peuvent être mis à la disposition de lecteurs intéressés qui voudraient en faire l'analyse, et ce, pour une période de 3 mois à compter de la date de parution de la liste. Pour plus de renseignements, veuillez écrire au rédacteur des Comptes rendus.

CORRECTIONS TO RECENT PUBLICATIONS
CORRECTIONS SUR RÉCENTES PUBLICATIONS

VOLUME 36, No. 6 — note by L. Chatarpaul, J. B. Robinson, and N. K. Kaushik: *Role of tubificid worms on nitrogen transformations in stream sediment.*

PAGE 676: Line 1 of the caption for Figure 3 should read "Fig. 3. Changes in the nitrate-N concentration in aerated".

VOLUME 36, No. 10 — paper by D. D. Lemon, P. H. LeBlond, and T. R. Osborn: *Seiche excitation in Port San Juan, British Columbia.*

PAGE 1224: Right column, line 4 "by tape changes, every 6 h until" should read "by tape changes every 6 h, until"; PAGE 1226: Right column, line 24 "exp(ivt)" should be "exp($i\nu t$)"; PAGE 1227: Right column, Guza and Davis (1974) reference, line 1 "Escitation" should be "Excitation".

COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Foreword to the special issue on the Experimental Lakes Area/Avant-propos au numéro spécial sur la région des lacs expérimentaux **J. Watson**

Evolution of the Experimental Lakes project **D. W. Schindler**

Hypolimnion injection of nutrient effluents as a method for reducing eutrophication **D. W. Schindler, T. Ruszczynski, and E. J. Fee**

Effects of a windstorm and forest fire on chemical losses from forested watersheds and on the quality of receiving streams **D. W. Schindler, R. W. Newbury, K. G. Beaty, J. Prokopowich, T. Ruszczynski, and J. A. Dulton**

Water renewal efficiency of watershed and lake combinations in the ELA region of the Precambrian Shield **R. W. Newbury and K. G. Beaty**

Experimental acidification of Lake 223, Experimental Lakes Area: background data and the first three years of acidification **D. W. Schindler, R. Wagemann, R. B. Cook, T. Ruszczynski, and J. Prokopowich**

Effects of artificial acidification on the growth of periphyton **P. Müller**

Decreased survival and calcium uptake by the crayfish *Oronectes virilis* in low pH **D. F. Malley**

Effects of acidification on mobilization of heavy metals and radionuclides from the sediments of a freshwater lake **D. W. Schindler, R. H. Hesslein, R. Wagemann, and W. S. Broecker**

Fates of metal radiotracers added to a whole lake: sediment–water interactions **R. H. Hesslein, W. S. Broecker, and D. W. Schindler**

Experimental study of trace metal chemistry in soft-water lakes at different pH levels **T. A. Jackson, G. Kipphut, R. H. Hesslein, and D. W. Schindler**

Dynamics of cadmium-stressed plankton communities **J. S. Marshall and D. L. Mellinger**

Experimental studies on the effect of arsenic on microbial degradation of organic matter and algal growth **G. J. Brunskill, B. W. Graham, and J. W. M. Rudd**

Continuous culturing of natural phytoplankton communities in the Experimental Lakes Area: effects of enclosure, in situ incubation, light, phosphorus, and cadmium **F. deNoyelles Jr., R. Knoechel, D. Reinke, D. Treanor, and C. Altenhofen**

Analysis of the response of hypolimnetic phytoplankton in continuous culture to increased light or phosphorus using track autoradiography **R. Knoechel and F. deNoyelles Jr.**

Physiological indicators of nutrient deficiency in lake phytoplankton **F. P. Healey and L. L. Hendzel**

Whole-lake radiocarbon experiment in an oligotrophic lake at the Experimental Lakes Area, northwestern Ontario **R. H. Hesslein, W. S. Broecker, P. D. Quay, D. W. Schindler**

Gas exchange, photosynthetic uptake, and carbon budget for a radiocarbon addition to a small enclosure in a stratified lake **P. Bower and D. McCorkle**

Errors in molybdenum blue methods for determining orthophosphate in freshwater **M. P. Stainton**

Radiochemical analysis of orthophosphate concentrations and seasonal changes in the flux of orthophosphate to seston in two Canadian Shield lakes **S. N. Levine and D. W. Schindler**

Comparison of the nitrogen-15 uptake and acetylene reduction methods for estimating the rates of nitrogen fixation by freshwater blue-green algae **B. M. Graham, R. D. Hamilton, and N. E. R. Campbell**

Nitrogen fixation in Canadian Precambrian Shield lakes **R. J. Flett, D. W. Schindler, R. D. Hamilton, and N. E. R. Campbell**

Denitrification in Lake 227 during summer stratification **Y. K. Chan and N. E. R. Campbell**

Important factors for estimating annual phytoplankton production in the Experimental Lakes Area **E. J. Fee**

Relationships between dipteran emergence and phytoplankton production in the Experimental Lakes Area, northwestern Ontario **I. J. Davies**

Comparison of fin-ray and scale age determinations for lake whitefish (*Coregonus clupeaformis*) and their implications for estimates of growth and annual survival **K. H. Mills and R. J. Beamish**

In situ measurements of pore water diffusion coefficients using tritiated water **R. H. Hesslein**

Whole-lake model for the distribution of sediment-derived chemical species **R. H. Hesslein**

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Recent Studies

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles on investigations published in other periodicals, which henceforth may be obtained in reprint form only from the author or originating establishment.

Récentes études

Jusqu'en 1970, ces articles étaient réimprimés, puis reliés dans un volume annuel d'études, comprenant deux parties. Les études de 1969, partie 2, incluant le n° 1355, ont été les dernières à être reliées. Nous n'en faisons désormais plus la reliure ni la distribution. Nous continuons cependant de numéroter les articles et d'en établir des listes d'études sur des recherches mentionnées dans d'autres périodiques. On peut donc se les procurer, sous leur forme réimprimée seulement, de l'auteur ou du service dépositaire.

2616. GERACI, J. R., AND T. G. SMITH. 1979. Vitamin C in the diet of Inuit hunters from Holman, Northwest Territories. Arctic 32(2): 135–139. (5)

2617. PAYNE, J. F., AND N. MAY. 1979. Further studies on the effect of petroleum hydrocarbons on mixed-function oxidases in marine organisms. Am. Chem. Soc. 99(20): 339–347, 1979. (9)

2618. SCHINDLER, D. W. 1978. Predictive eutrophication models. Limnol. Oceanogr. 23: 1080–1081. (4)

2619. CARLANDER, K. D., J. S. CAMPBELL, AND R. J. MUNCY. 1978. Inventory of percid and esocid habitat in North America. Am. Fish. Soc. Spec. Publ. 11: 17–38. (4)

2620. CAUVIN, D. M. 1978. The allocation of resources in fisheries: an economic perspective. Am. Fish. Soc. Spec. Publ. 11: 361–370. (4)

2621. LI, M. F., AND S. CLYBURNE. 1979. Mortalities of blue mussel (*Mytilus edulis*) in Prince Edward Island. J. Invertebr. Pathol. 33: 108–110. (8)

2622. SERGEANT, D. E., AND K. HAY. 1979. Migratory sea mammals populations in Lancaster Sound, p. 1–31. Canada, Dept. Indian and Northern Affairs, Environmental–Social Program, ESCOM Rep. No. AI-21. (5)

2623. SMITH, T. G., K. HAY, D. TAYLOR, AND R. GREENDALE. 1979. Ring seal breeding habitat in Viscount Melville Sound, Barrow Strait and Peel Sound, p. 1–85. Canada, Dept. Indian and Northern Affairs, Environmental–Social Program ESCOM Rep. No. AI-22. (5)

2624. PATERSON, W. D., AND J. E. STEWART. 1979. Rate and duration of phagocytic increase in lobsters induced by Pseudomonas perolens endotoxin. Dev. Comp. Immun. 3: 353–357. (8)

2625. SOLOMON, J. 1979. Sample cleanup and concentration apparatus for the determination of chlorinated hydrocarbon residues in environmental samples. Anal. Chem. 51: 1861–1863. (4)

2626. SILVERT, W. 1979. Practical curve fitting. Limnol. Oceanogr. 24: 767–773. (7)

2627. GILGAN, M. W., AND B. G. BURNS. 1979. Ecdysterone triacetate-induced molting in the American lobster (*Homarus americanus*). Comp. Biochem. Physiol. 64A: 125–131. (8)

2628. YURKOWSKI, M., AND J. L. TABACHEK. 1978. Proximate and amino acid composition of some natural fish foods, p. 435–448. *In* Proc. World Symp. on Finfish Nutrition and Fishfeed Technology, Hamburg 20–23 June, Vol. I. Berlin 1979. (4)

2629. POVOLEDO, D., AND M. PITZE. 1979. Preparation of low-ash humic acids, and the accompanying losses of organic material, from Canadian lake sediments and a gleysol. Soil Sci. 128: 1–8. (4)

2630. BARCIA, J. 1979. Massive fish mortalities caused by algal blooms in eutrophic ecosystems. Symp. Biol. Hung. 19: 121–124. (4)

2631. MCLEESE, D. W., AND C. D. METCALFE. 1979. Toxicity of creosote to larval and adult lobsters and crangon and its accumulation in lobster hepatopancreas. Bull. Environ. Contam. Toxicol. 22: 796–799. (6)

2632. MCLEESE, D. W., AND C. D. METCALFE. 1979. Toxicity of mixtures of phosphamidon and methidathion to lobsters (*Homarus americanus*). Chemosphere (2): 59–62. (6)

2633. MCLEESE, D. W., V. ZITKO, AND M. R. PETERSON. 1979. Structure-lethality relationships for phenols, anilines and other aromatic compounds in shrimp and clams. Chemosphere (2): 53–57. (6)

2634. PETERSON, R. H., AND J. L. METCALFE. 1979. Responses of Atlantic salmon alevins to temperature gradients. Can. J. Zool. 57: 1424–1430. (6)

2635. RAY, S. N., AND W. J. WHITE. 1979. *Equisetum arvense* — an aquatic vascular plant as a biological monitor for heavy metal pollution. Chemosphere (5): 125–128. (6)

2636. WHITE, A. W. 1978. Salinity effects on growth and toxin content of *Gonyaulax excavata*, a marine dinoflagellate causing paralytic shellfish poisoning. J. Phycol. 14: 475–479. (6)

2637. ZITKO, V., AND R. L. SAUNDERS. 1979. Effect of PCB's and other organochlorine compounds on the hatchability of Atlantic salmon (*Salmo salar*) eggs. Bull. Environm. Contam. Toxicol. 21: 125–130. (6)

2638. ZITKO, V., D. W. MCLEESE, C. D. METCALFE, AND W. G. CARSON. 1979. Toxicity of permethrin, decamethrin, and related pyrethroids to salmon and lobster. Bull. Environm. Contam. Toxicol. 21: 338–343. (6)

2639. ZITKO, V. 1979. PCB levels in human adipose tissue and population density. Chemosphere (2): 45–46. (6)

2640. ZITKO, V. 1979. An equation of lethality curves in

tests with aquatic fauna. Chemosphere (2): 47–51. (6)

2641. JYRKKANEN, J., AND D. G. WRIGHT. 1979. First record of the northern brook lamprey, *Ichthyomyzon fossor*, in the Nelson River drainage, Manitoba. Can. Field Nat. 93: 199–200. (4)

2642. FRIESEN, M. K., J. F. FLANNAGAN, AND S. G. LAWRENCE. 1979. Effects of temperature and cold storage on development time and viability of eggs of the burrowing mayfly *Hexagenia rigida* (Ephemeroptera: Ephemeridae). Can. Entomol. 111: 665–673. (4)

2643. DRINKWATER, K., B. PETRIE, AND W. H. SUTCLIFFE JR. 1979. Seasonal geostrophic volume transports along the Scotian shelf. Estuarine Coastal Mar. Sci. 9: 17–27. (7)

2644. BRUNDIN, L., AND O. A. SÆTHER. 1978. *Buchonomyia burmanica* sp.n. and Buchnonomyiinae, a new subfamily among the chironomidae (Diptera). Zool. Ser. 7: 269–275. (4)

2645. FEE, E. J. 1979. A relation between lake morphometry and primary productivity and its use in interpreting whole-lake eutrophication experiments. Limnol. Oceanogr. 24: 401–416. (4)

2646. DUANGSAWASDI, M., AND J. F. KLAVERKAMP. 1979. Acephate and fenitrothion toxicity in rainbow trout: effects of temperature stress and investigations on the sites of action, p. 35–51. *In* L. L. Marking and R. A. Kimerle [ed.] Aquatic toxicology, ASTM STP 667, American Society for Testing and Materials. (4)

2647. WHITE, A. W. 1979. Dinoflagellate toxins in phytoplankton and zooplankton fractions during a bloom of Gonyaulax excavata, p. 381–384. *In* Taylor/Seliger [ed.] Toxic dinoflagellate blooms, Elsevier North Holland, Inc. (6)

2648. AIKEN, D. E., AND S. L. WADDY. 1978. Space density and growth of the lobster (*Homarus americanus*), p. 461–467. *In* J. W. Avault Jr. [ed.] Proc. Ninth Ann. Meeting World Mariculture Society. (6)

2649. HOLMBERG, B., AND R. L. SAUNDERS. 1979. The effects of pentachlorophenol on swimming performance and oxygen consumption in the American eel (*Anguilla rostrata*). Rapp. P.-V. Reun. Cons. Int. Explor. Mer. 174: 144–149.

2650. ROSENBERG, D. M., H. V. DANKS, J. A. DOWNES, A. P. NIMMO, AND G. E. BALL. 1979. 47. Procedures for a faunal inventory, p. 509–532. *In* H. V. Danks [ed.] Approaches to an exploration of the Canadian fauna, Canada and its insect fauna. Mem. Ent. Soc. Can. 108: 573 p. (4)

2651. MCLEESE, D. W., V. ZITKO, AND D. B. SERGEANT. 1979. Uptake and excretion of fenitrothion by clams and mussels. Bull Environm. Contam. 22: 800–806. (6)

2652. RANTALA, R. T. T., AND D. H. LORING. 1978. Atomic absorption analysis of USGS reference sample marine mud MAG-1 for selected trace elements. Geostandards Newsletter 2: 125–127. (7)

2653. ANTIA, N. J., AND R. S. KRIPPS. 22: Threonine deaminase in marine microalgae, p. 226–232. *In* J. A. Hellebust and J. S. Craigie [ed.] Handbook of phycological methods, physiological and biochemical methods ch. 1, Cambridge Univ. Press, London.

2654. HARGRAVE, B. T., AND G. A. PHILLIPS. 1977. Oxygen uptake of microbial communities on solid surfaces, p. 545–587. *In* J. Cairns [ed.] Aquatic Microbial Communities Ch. 17, Garland Publ. New York, N.Y. (7)

Translation Series

The following translations have been prepared by or for the Department of Fisheries and Oceans for use in its investigations. Copies are available from Translation Services, Canada Institute for Scientific and Technical Information, National Research Council of Canada, Ottawa, Ontario K1A 0S2, at a cost of 22¢ per page and a minimum charge of $2.20. Information on translations can be obtained from the Scientific Information and Publications Branch.

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4526. LU, Y., AND J. WANG. 1978. Tides and their relationship with weather. Hai-yang wen-xuan (1): 16–24, 1978. (Translated from Chinese by Fish. Mar. Serv. Transl. Ser. No. 4526, 1979).

4527. AVDEYEV, I. I., V. N. AKULIN, AND N. A. ROMASHINA. 1976. Change in the lipid composition of the sardine and boarfish during frozen storage. Issled. Po. Tekhnol. Ryb. Produktov. (6): 20–39, 1976. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 4527, 1979).

4528. KANAMARU, S. [undated]. The life of paroctopus hongkongensis and the species of octopuses along the Rumoi Coast, Hokkaido. Hokkaido Suisan Shikenjo Geppo, p. 189–209. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 4528, 1979).

4529. SIEBECK, O. 1968. The "avoidance of shore" and the optical orientation of pelagic crustaceans. Arch. Hydrobiol. Suppl. 35(1): 40–112. (Translated from German by Fish. Mar. serv. Transl. Ser. No. 4529, 1979).

4530. SHCHEKATURINA, T. L. 1977. Methods and results of the fractional determination of the lipids of black sea mussels in the process of their decomposition. Biol. Morya. Resp. Mezhved. Sb. (41): 91–95, 1977. (Translated from Russian by Fish. Mar. Serv. Transl. Ser. No. 4530, 1979).

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ANNOUNCEMENTS / AVIS

First Call for Papers, 1980 Annual Meeting

The 1980 Annual Meeting (110th) of the American Fisheries Society will be held at the Galt House, Louisville, Kentucky, September 21–24, 1980. The meeting will be held in cooperation with and overlapping the annual meeting of the International Association of Fish and Wildlife Agencies. Contributed technical paper sessions will be emphasized, but proposals for symposia, modules, debates and poster sessions to be convened by individuals or groups will be accepted until January 31, 1980.

The deadline for submission of formal abstracts to be considered for contributed paper sessions is March 15, 1980. Abstracts should be double-spaced on plain paper and limited to a maximum of 250 words. The abstract should include the reason for the study, the objectives, and the principal results and conclusions. Each abstract should have attached a title and the full name and address of each author. Also, indicate if any of the authors is a student, and what visual aid requirements will be needed. Authors will be notified of the status of their submission by late May.

The visual aids used with presentations will be held to a high standard and are subject to approval by the Program Committee in advance. Authors of accepted papers will receive further instructions on visual aid requirements.

All proposals, abstracts, and queries should be sent to *H. Ronald Preston, AFS Program Chairman 1980, 303 Methodist Building, 11th and Chapline Streets, Wheeling, West Virginia 26003, USA.*

Premier appel d'articles — Réunion annuelle de 1980

La réunion annuelle de 1980 (la 110e) de l'American Fisheries Society se tiendra à Galt House, Louisville, au Kentucky, du 21 au 24 septembre 1980. La réunion se tiendra en coopération avec et chevauchera celle de l'International Association of Fish and Wildlife Agencies. Comme par le passé on mettra l'accent sur des séances au cours desquelles seront présentés des travaux techniques, mais on acceptera jusqu'au 31 janvier 1980 des suggestions sur colloques, modules, débats, panneaux-réclames et autres séances convoquées par des particuliers ou des groupes.

Les résumés dans les formes pour les séances consacrées aux contributions devront être soumis pour considération avant le 15 mars 1980. Ces résumés devront être tapés à double espace sur papier ordinaire et limités à un maximum de 250 mots. Ils devront inclure la raison de l'étude, les objectifs et les principaux résultats et conclusions. Chaque résumé devra avoir un titre et contenir le nom au complet et l'adresse de chaque auteur. Indiquez de plus si l'auteur est un étudiant et mentionnez le genre d'aide visuelle requis. Les auteurs seront avisés vers fin mai de l'état de leur soumission.

On s'assurera que les aides visuelles accompagnant les présentations soit d'un haut calibre et elles seront sujettes à l'approbation du comité du programme à l'avance. Les auteurs dont les travaux auront été acceptés recevront des instructions supplémentaires concernant les aides visuelles.

Suggestions, résumés et demandes de renseignements devront être adressés à *H. Preston, AFS Program Chairman 1980, 303 Methodist Building, 11th and Chapline Streets, Wheeling, West Virginia 26003, É.-U.*

BULLETIN 200

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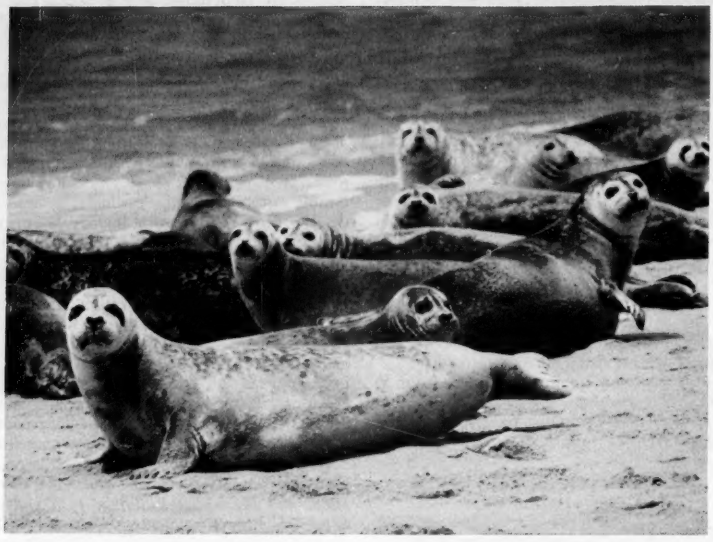

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